The American Journal of Human Genetics

Vol. 11 No. 2 *June 1959*

Huntington's Chorea in Michigan[1,2]

2. Selection and Mutation

T. EDWARD REED AND JAMES V. NEEL

Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan

INTRODUCTION

THIS PAPER is the second in a series of three describing the results of a comprehensive study of Huntington's chorea in the state of Michigan. The first paper was concerned with the demography and certain aspects of the genetics of this disease (Reed and Chandler, 1958). This presentation will be concerned with selection and mutation in relation to this condition. The third and final communication will be of a clinical nature.

Previous data have suggested that the relative fitness of choreics, compared to normal persons, is in the neighborhood of unity or above (Panse, 1942; S. Reed and Palm, 1951). If this were in fact the case, it would be a rare—not to say unique—situation in human genetics: individuals affected with a severely debilitating disease whose onset is often during the reproductive period nevertheless actually achieving a greater-than-normal fertility. Also, if these studies are correct, it then becomes necessary to explain why Huntington's chorea is a rare disease today, having a frequency in populations of European ancestry of about 4×10^{-5}. This study was undertaken primarily to analyze the population "dynamics" of the gene responsible for this disease in the state of Michigan. It will be shown that not only are individuals heterozygous for this gene at a reproductive disadvantage as compared with normal, but that there are probably at least three and possibly four different ways in which this disadvantage is brought about. The unreliability of sibling controls in studies of genetically determined fertility differentials will be demonstrated for the case of Huntington's chorea. Finally, the rate with which mutation resulting in this phenotype occurs will be calculated.

SELECTION

The essence of this paper is an attempt to estimate the fitness of individuals with Huntington's chorea and to utilize this estimate in several calculations. Estimating fitness is always difficult in man and is especially so for Huntington's chorea. For this reason it is necessary to give considerable attention to the methods which were used.

Received July 5, 1958.

[1] This study was supported in part by contract AT(11-1)-405 of the United States Atomic Energy Commission.

[2] We wish to acknowledge the assistance on clinical aspects of this study given by Dr. Joseph H. Chandler and the excellent field work done by Mrs. Estella Hughes and Mrs. Ruth Davidson.

A. Methodology

The fitness of one class of humans, say population *1* (P_1), relative to that of another, P_2, has usually been defined as $\bar{B}_1/\bar{B}_2$, where the $\bar{B}$'s refer to mean fertility. There are a variety of ways in which the mean fertility may be estimated and a number have been used, some without apparent genetic justification. Reed (1959) has recently discussed the definitions of relative fitness (W) for human populations and proposed the following definition as best: Assume that for P_1 and P_2 we have complete information on the survival and reproduction of a large random sample of liveborn individuals who have been followed from birth to death. Let

x = age in years at last birthday
N_i = original number of newborn liveborn individuals of population i
$B_{i\cdot x}$ = number of livebirths born to the survivors of the N_i individuals during their xth year of life
$\bar{B}_{i\cdot o}$ = mean number of livebirths ever born per newborn liveborn individual of P_i
$B_{i\cdot y}$ = mean number of livebirths ever born per newborn liveborn individual of P_i per year, based on $\bar{B}_{i\cdot o}$ livebirths ever born
$P_{i\cdot x}$ = parental age frequency distribution of the N_i individuals (the proportion of livebirths, out of $\bar{B}_{i\cdot o}$ livebirths, which is born to the survivors of these individuals at age x).

Then,

$$\bar{B}_{i\cdot o} = \frac{1}{N_i}\sum_x B_{i\cdot x}\,,\quad P_{i\cdot x} = \frac{B_{i\cdot x}}{\sum_x B_{i\cdot x}}\,,\quad B_{i\cdot y} = \frac{1}{N_i}\sum_x \frac{B_{i\cdot x}}{x}\,,$$

and the proposed definition of W for populations *1* and *2*, is

$$W = \frac{B_{1\cdot y}}{B_{2\cdot y}} \tag{1}$$

or its equivalent

$$W = \frac{\bar{B}_{1\cdot o}\sum_x \frac{P_{i\cdot x}}{x}}{\bar{B}_{2\cdot o}\sum_x \frac{P_{2\cdot x}}{x}}. \tag{2}$$

The number of livebirths ever born per newborn is used in order to include all factors affecting genetic fitness: viability, marriage, adult fertility. $B_{i\cdot y}$ is used instead of $\bar{B}_{i\cdot o}$ alone because the latter is in reality a mean number of livebirths *per generation* and we have no assurance that the generation lengths of P_1 and P_2 are equal. Even if equal, the mean number of livebirths ever (to be) born per newborn *per year*, which gives an exact rate of increase of the population due to births, may differ since this is proportional to $\sum_x \frac{P_{i\cdot x}}{x}$. When the parental age distributions are the same, then, as (2) shows, (1) reduces to the usual ratio of means, $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$.

One further point should be noted about what may be called the reference popula-

tion, P_2. Unless otherwise specified, the fitness of a group or genotype should always be related to the general population. The use of other groups, such as normal sibs, for P_2 may be allowed for convenience or necessity but it then becomes necessary to show that P_2 is representative of the general population in its fertility. As will be seen, the non-choreic sibs of the choreic individuals of the present study differ significantly in their completed fertility from the general population of Michigan. Consequently, a direct comparison with sibs would give a misleading estimate. It was therefore necessary to use several different approaches to obtain valid comparisons of choreics (or heterozygotes) with the general population.

Bias due to ascertainment of the kindreds (i.e., obtaining an unrepresentative sample) is believed to be negligible in this study since ascertainment is almost complete, sibships being used as of 1940 and ascertainment having continued until 1956.

In all the calculations to follow, the fitnesses of males and females were calculated separately to test whether there are significant differences. Such a difference has already been found for one dominant trait, namely neurofibromatosis (Crowe, Schull, and Neel, 1956).

In addition to the difficulties in estimating fitness discussed above, there is another peculiar to Huntington's chorea and other dominant traits with delayed onset, namely, the inability, at any specified age, to recognize a certain fraction of heterozygotes. Since the "normal" non-choreic sibs of choreics may still, at any age, in fact be heterozygotes who have failed to manifest the disease, and their children may receive the gene for Huntington's chorea and develop the disease themselves, it is necessary to estimate the number of non-choreic heterozygotes by the use of age-onset curves. This number, added to the number of choreics, gives an estimate of the total number of heterozygotes. It is the fitness of heterozygotes, not choreics, which is of *genetic* importance. The fitness of choreics is, of course, of interest for other reasons. The necessity for this correction, i.e., estimating the number of heterozygotes from the number of trait-bearers, has sometimes been overlooked.

Possible differences in the survival from birth to age five years, between children of choreics and children of non-choreics, were also investigated, since Panse (1942) has found evidence for increased mortality among children of choreics.

B. Results

1. *Sibship comparisons of mean number of children ever born*

For a series of choreics and their non-choreic siblings the conventional ratio of mean numbers of children was calculated, subject to the conditions discussed above. The sibships used are those containing a Michigan choreic living on April 1, 1940, all members of the sibship being used. The status of each member is taken as of the time of death or, if living, the time of last investigation (1954 to 1956). The classification of "choreic" means that either a) a person has been medically diagnosed as having Huntington's chorea (see Part 1 of this study) and/or b) he has been so classified by reliable, non-contradictory lay reports. If only b), he is also a near biological relative (sib, son, etc.) of a medically diagnosed choreic. It is not desirable to restrict "choreic" to medically diagnosed cases since this introduces selection for severity of the disease and increased age of the individual. "Non-choreic" means that there is reliable in-

formation that the individual in question, at the time of investigation, lacked signs of the trait. The three sibships containing "Negro" choreics were excluded as well as all sibships in which information on fertility or choreic status was faulty or lacking. Sibships in which a choreic was ascertained only through an affected descendant, were omitted. The need to estimate the number of non-choreic heterozygotes imposed further restrictions. This was met by calculating the probability C_x that a non-choreic sib of a choreic has the gene for Huntington's chorea but does not show the trait at age x, given that his parent has the gene. Therefore sibships for which one parent could not be considered to be heterozygous for the gene leading to Huntington's chorea (*Hh*) were omitted.

In order to ensure that the reproduction of individuals was nearly, or completely, terminated, only persons living at age 45 or over, or deceased at age 15 or over, were used. Therefore choreics and non-choreics who die relatively young are included in the survey. The age 45 is a compromise between the desire to be certain that reproduction of the still-living is completed and the desire not to select for mildly affected choreics who can live to advanced ages. Age 15 was chosen because no chorea occurred earlier in this sample. Therefore the assumption that the gene has no effect on the fitness of its bearer before age 15 seems plausible, although, of course, this is not certain. This lower age limit enables us to neglect, for this calculation, the many infant and juvenile deaths which, since we cannot determine genotype, contribute no information. After making these omissions, 120 choreic males, 137 choreic females, 97 non-choreic males and 113 non-choreic females were available for study. Five individuals could not be classified either as "choreic" or "non-choreic" from available information. They were omitted from the calculations since preliminary work (Reed, 1957) showed that this omission had a negligible effect.

In order to estimate C_x, we may note that if P_x is the probability that an individual who is *Hh* develops chorea by age x, then

$$C_x = \frac{1 - P_x}{2 - P_x}.$$

P_x may be estimated from the age of onset distribution as was done in Part 1 of this study. Since the values of C_x may be of interest elsewhere, they are presented, with the values for P_x, in Table 1. It may be noted that at age 40, C_x is 0.240 so that the assumption that non-choreic sibs of this age are *hh* (homozygous normal) would lead to gross error. At age 50, C_x becomes 0.063, and at 60, 0.006. C_x at advanced ages is perhaps unreliable because the apparently normal distribution of ages of onset may not, in fact, obtain for extreme deviations from the mean.

In Tables 2 and 3 the distribution by age at death or the age at time of last investigation of the above-described choreic individuals and their non-choreic sibs are presented, together with the number of liveborn children ever born to them. Tables 4 and 5 give the distribution by marital status of the number of liveborn children ever born to these individuals. From the distributions in Tables 2 and 3 the number N of heterozygotes among the non-choreic sibs was calculated from the relation

$$N = \sum_x N_x C_x$$

TABLE 1. VALUES OF P_x AND C_x. (SEE TEXT FOR DEFINITION.) P_x VALUES DERIVED FROM TABLE 15 OF PART 1 OF THIS STUDY, ASSUMING AGE OF ONSET IS NORMALLY DISTRIBUTED WITH MEAN 35.30 YEARS AND STANDARD DEVIATION 9.80 YEARS

Age x	P_x	C_x	Age x	P_x	C_x	Age x	P_x	C_x	Age x	P_x	C_x
15	.019	.495	30	.295	.413	45	.839	.139	60	.994	.006
16	.024	.494	31	.330	.401	46	.862	.121	61	.996	.004
17	.031	.492	32	.367	.388	47	.883	.105	62	.997	.003
18	.038	.490	33	.405	.373	48	.903	.088	63	.998	.002
19	.048	.488	34	.448	.356	49	.919	.075	64	.998	.002
20	.059	.485	35	.488	.339	50	.933	.063	65	.999	.001
21	.072	.481	36	.528	.321	51	.945	.052	66	.999	.001
22	.087	.477	37	.567	.302	52	.955	.043	67	.999	.001
23	.104	.473	38	.610	.281	53	.965	.034	68	1.000	$<.0005$
24	.125	.467	39	.648	.260	54	.972	.027			
25	.147	.460	40	.684	.240	55	.978	.022			
26	.171	.453	41	.719	.219	56	.983	.017			
27	.198	.445	42	.752	.199	57	.986	.014			
28	.227	.436	43	.785	.177	58	.990	.010			
29	.261	.425	44	.813	.158	59	.992	.008			

where N_x is the number of non-choreics age x at time of death or last investigation. The number B of liveborn children ever born to these N non-choreic heterozygotes is

$$B = \sum_x B'_x C_x$$

where B'_x is the number of livebirths born to non-choreics who were age x at time of death or last investigation, assuming that the fertility of non-choreic heterozygotes is the same as that of all non-choreics. If one makes the more extreme assumption that the fertility of these non-choreic heterozygotes is the same as that of all choreics, the mean is only slightly changed, dropping about 0.005 in the males and increasing about 0.026 in the females. In this situation it seems adequate to make the former assumption. The values of N and B for all groups, together with the numbers of individuals and livebirths and the means for all groups, are given in Table 6.

About ten per cent of the non-choreic sibs in this sample are calculated to be heterozygotes. The number of these heterozygotes plus the number of choreics give the estimated number of heterozygotes in each category. We note that the mean for all non-choreic males, 2.072 ± 0.260, does not differ from the mean for all non-choreic females, 2.027 ± 0.202, so that males and females may be pooled to give a better estimate of the fertility of non-choreics. There is, however, a marked and significant difference in the fertility of male choreics and female choreics, the former being 1.850 ± 0.198, the latter 2.818 ± 0.233. A large fraction of this difference is explainable by the relatively high proportion of male choreics who fail to marry. In Tables 4 and 5 we see that the proportion of "never married" male choreics is $31/120 = 0.258$, while the corresponding proportion for female choreics is $11/137 = 0.080$.

TABLE 2. DISTRIBUTION BY AGE AT DEATH OF THE NUMBER OF DECEASED CHOREICS AND DECEASED NON-CHOREIC SIBS, AND THE NUMBER OF LIVEBORN CHILDREN EVER BORN TO THEM. SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940 AND SELECTED, AS DESCRIBED IN TEXT, FOR ESTIMATING RELATIVE FITNESS. N_x = NUMBER OF INDIVIDUALS AGE x; B'_x = NUMBER OF LIVEBIRTHS TO INDIVIDUALS AGE x.

Age x	Males				Females				Age x	Males				Females			
	Choreic		Non-Choreic		Choreic		Non-choreic			Choreic		Non-choreic		Choreic		Non-choreic	
	N_x	B'_x	N_x	B'_x	N_x	B'_x	N_x	B'_x		N_x	B'_x	N_x	B'_x	N_x	B'_x	N_x	B'_x
15	0	0	1	0	0	0	0	0	50	6	9	0	0	0	0	1	10
16	0	0	0	0	0	0	0	0	51	3	7	2	4	9	40	1	6
17	0	0	0	0	0	0	2	0	52	4	8	0	0	4	12	0	0
18	0	0	1	0	0	0	0	0	53	5	12	1	3	7	15	1	0
19	0	0	1	0	0	0	0	0	54	2	6	0	0	4	11	0	0
20	0	0	0	0	0	0	0	0	55	3	2	0	0	7	29	1	4
21	0	0	0	0	0	0	1	0	56	3	7	0	0	2	0	0	0
22	1	0	1	0	0	0	1	0	57	2	7	3	14	1	2	0	0
23	0	0	2	0	1	1	1	0	58	1	2	0	0	2	4	3	5
24	0	0	3	1	0	0	2	0	59	1	7	1	4	1	0	1	2
25	0	0	0	0	0	0	0	0	60	1	3	0	0	3	14	1	0
26	1	0	0	0	1	0	1	1	61	5	9	1	0	0	0	1	0
27	1	0	0	0	0	0	2	1	62	0	0	2	10	5	23	1	0
28	0	0	0	0	0	0	1	0	63	3	6	1	0	3	4	0	0
29	0	0	3	2	0	0	1	0	64	5	19	1	2	3	11	0	0
30	1	0	1	0	2	1	3	6	65	2	3	3	15	0	0	0	0
31	2	0	0	0	2	2	1	3	66	0	0	0	0	1	4	0	0
32	0	0	0	0	0	0	1	2	67	3	4	1	1	3	3	0	0
33	1	0	0	0	0	0	1	5	68	0	0	0	0	1	6	1	2
34	0	0	2	4	0	0	1	0	69	1	0	2	7	2	9	1	0
35	1	2	0	0	1	2	0	0	70	2	2	1	0	0	0	2	3
36	1	5	0	0	1	2	0	0	71	0	0	1	0	1	0	0	0
37	5	9	0	0	2	4	0	0	72	0	0	1	2	0	0	0	0
38	1	0	0	0	0	0	0	0	73	2	6	3	5	2	2	1	2
39	1	4	1	0	2	0	3	3	74	0	0	0	0	2	4	0	0
40	2	6	1	0	3	5	2	5	75	1	0	2	4	1	4	1	0
41	2	0	0	0	1	9	0	0	76	0	0	1	7	1	12	1	3
42	2	3	0	0	1	0	0	0	77	1	3	1	1	0	0	1	8
43	1	0	0	0	2	11	1	0	78	1	1	1	0	2	9	0	0
44	3	5	0	0	2	3	1	4	79	0	0	0	0	0	0	1	4
45	6	5	1	0	4	9	0	0	80	1	0	0	0	0	0	0	0
46	1	0	1	0	4	12	2	4	81	0	0	0	0	0	0	0	0
47	3	2	0	0	1	3	0	0	82	0	0	0	0	0	0	1	6
48	1	0	2	11	5	13	0	0	83	0	0	0	0	0	0	0	0
49	2	1	0	0	5	17	0	0	84	0	0	1	1	0	0	0	0
									85	0	0	0	0	0	0	1	4
									Total	97	165	51	98	107	312	50	93

TABLE 3. DISTRIBUTION BY AGE AT TIME OF LAST INVESTIGATION OF THE NUMBER OF LIVING CHOREICS AND LIVING NON-CHOREIC SIBS AND THE NUMBER OF LIVEBORN CHILDREN EVER BORN TO THEM. SIBSHIPS CONTAINING A MICHIGAN CHOREIC ON APRIL 1, 1940 AND SELECTED, AS DESCRIBED IN TEXT, FOR ESTIMATING RELATIVE FITNESS. N_x AND B'_x AS IN TABLE 2.

Age x	Males				Females			
	Choreic		Non-choreic		Choreic		Non-choreic	
	N_x	B'_x	N_x	B'_x	N_x	B'_x	N_x	B'_x
45	0	0	3	7	1	2	1	0
46	0	0	1	1	2	6	7	23
47	0	0	2	0	0	0	0	0
48	2	4	1	1	3	9	1	4
49	2	3	0	0	1	7	3	4
50	0	0	3	2	0	0	3	4
51	3	4	2	5	1	1	2	6
52	0	0	2	2	2	5	1	1
53	1	3	0	0	1	2	2	6
54	2	7	2	2	1	0	0	0
55	0	0	2	6	0	0	2	7
56	1	1	0	0	2	9	2	6
57	0	0	0	0	1	2	2	0
58	2	4	1	4	3	1	3	4
59	0	0	0	0	1	0	2	7
60	1	0	1	0	0	0	1	2
61	1	0	4	15	2	4	1	3
62	2	12	2	10	1	4	1	0
63	1	2	0	0	1	4	1	1
64	1	8	1	0	0	0	5	14
65	1	0	4	8	0	0	0	0
66	1	2	0	0	0	0	2	4
67	0	0	1	1	0	0	2	0
68	0	0	1	0	0	0	5	9
69	0	0	1	0	0	0	1	5
70	1	0	1	2	2	11	1	3
71	0	0	3	6	2	2	1	4
72	0	0	0	0	0	0	1	2
73	0	0	2	5	0	0	2	5
74	0	0	2	6	0	0	2	4
75	0	0	0	0	1	5	0	0
76	0	0	2	10	0	0	3	1
77	1	7	0	0	0	0	0	0
78	0	0	0	0	1	0	1	4
79	0	0	0	0	0	0	0	0
80	0	0	1	2	1	0	0	0
81	0	0	0	0	0	0	1	0
82	0	0	1	8	0	0	1	3
Total	23	57	46	103	30	74	63	136

TABLE 4. DISTRIBUTION OF NUMBER OF LIVEBORN CHILDREN EVER BORN TO THE MALES TABULATED IN TABLES 2 AND 3.
S = SINGLE (NEVER MARRIED); M = MARRIED (EVER MARRIED)

Number of Live-births	Choreic									Non-Choreic								
	Deceased ≥ 15			Living ≥ 45			Total			Deceased ≥ 15			Living ≥ 45			Total		
	S	M	Σ	S	M	Σ	S	M	Σ	S	M	Σ	S	M	Σ	S	M	Σ
0	27	13	40	3	4	7	30	17	47	15	8	23	6	11	17	21	19	40
1	1	12	13	0	3	3	1	15	16	0	7	7	0	3	3	0	10	10
2	0	14	14	0	5	5	0	19	19	0	7	7	0	6	6	0	13	13
3	0	17	17	0	2	2	0	19	19	0	4	4	0	10	10	0	14	14
4	0	4	4	0	2	2	0	6	6	0	5	5	0	2	2	0	7	7
5	0	2	2	0	1	1	0	3	3	0	0	0	0	4	4	0	4	4
6	0	4	4	0	0	0	0	4	4	0	0	0	0	1	1	0	1	1
7	0	2	2	0	1	1	0	3	3	0	1	1	0	1	1	0	2	2
8	0	0	0	0	1	1	0	1	1	0	1	1	0	1	1	0	2	2
9	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	2	2
10	0	0	0	0	1	1	0	1	1	0	1	1	0	0	0	0	1	1
11	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. of live-births	1	164	165	0	57	57	1	221	222	0	98	98	0	103	103	0	201	201
Total no. of individuals	28	69	97	3	20	23	31	89	120	15	36	51	6	40	46	21	76	97
Mean no. of live-births	0.036	2.377	1.701	0.000	2.850	2.478	0.032	2.483	1.850	0.000	2.722	1.922	0.000	2.575	2.239	0.000	2.645	2.072
Variance	0.036	3.944	3.941	0.000	7.818	7.715	0.032	4.775	4.700	0.000	8.549	7.554	0.000	5.481	5.519	0.000	6.845	6.547
S.E. of mean	0.036	0.239	0.202	0.000	0.625	0.579	0.032	0.232	0.198	0.000	0.487	0.385	0.000	0.370	0.346	0.000	0.300	0.260

S.E. of mean	0.036	0.239	0.202	0.000	0.625	0.579	0.032	0.232	0.198	0.000	0.487	0.385	0.000	0.370	0.346	0.000	0.300	0.260

TABLE 5. DISTRIBUTION OF NUMBER OF LIVEBORN CHILDREN EVER BORN TO THE FEMALES TABULATED IN TABLES 2 AND 3.
S = SINGLE (NEVER MARRIED); M = MARRIED (EVER MARRIED)

Number of Live-births	Choreic									Non-Choreic								
	Deceased ≥ 15			Living ≥ 45			Total			Deceased ≥ 15			Living ≥ 45			Total		
	S	M	Σ	S	M	Σ	S	M	Σ	S	M	Σ	S	M	Σ	S	M	Σ
0	9	13	22	0	8	8	9	21	30	11	12	23	9	8	17	20	20	40
1	1	17	18	1	1	2	2	18	20	0	3	3	0	9	9	0	12	12
2	0	22	22	0	7	7	0	29	29	0	8	8	0	14	14	0	22	22
3	0	13	13	0	3	3	0	16	16	0	6	6	0	9	9	0	15	15
4	0	8	8	0	6	6	0	14	14	0	4	4	0	7	7	0	11	11
5	0	2	2	0	2	2	0	4	4	0	2	2	0	2	2	0	4	4
6	0	8	8	0	0	0	0	8	8	0	2	2	0	2	2	0	4	4
7	0	5	5	0	1	1	0	6	6	0	0	0	0	2	2	0	2	2
8	0	4	4	0	1	1	0	5	5	0	1	1	0	1	1	0	2	2
9	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
10	0	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	1	1
11	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
12	0	2	2	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0
Total no. of live-births	1	311	312	1	73	74	2	384	386	0	93	93	0	136	136	0	229	229
Total no. of individuals	10	97	107	1	29	30	11	126	137	11	39	50	9	54	63	20	93	113
Mean no. of live-births	0.100	3.206	2.916	1.000	2.517	2.467	0.182	3.048	2.818	0.000	2.385	1.860	0.000	2.519	2.159	0.000	2.462	2.027
Variance	0.100	8.145	8.210	0.000	4.687	4.602	0.164	7.390	7.415	0.000	5.717	5.429	0.000	3.764	4.007	0.000	4.534	4.615
S.E. of mean	0.100	0.290	0.277	0.000	0.402	0.392	0.140	0.242	0.233	0.000	0.383	0.330	0.000	0.264	0.252	0.000	0.221	0.202

TABLE 6. NUMBER OF INDIVIDUALS, NUMBER OF LIVEBIRTHS, AND MEAN NUMBER OF LIVEBIRTHS
Data of Tables 2, 3, 4, and 5. Numbers for specified genotypes are estimated. See text

Hh = heterozygote for gene for Huntington's chorea
$(Hh)_n$ = non-choreic *Hh* individual
hh = homozygous normal individual
B = observed number of liveborn children ever born to the *N* individuals (age $\geq$ 15 years)
$\bar{B}_o$* = estimated mean number of liveborn children ever born per *newborn* individual
N = number of individuals

Status		Males					Females				
		Chore-ics	Non-chore-ics	$(Hh)_n$	*Hh*	*hh*	Chore-ics	Non-chore-ics	$(Hh)_n$	*Hh*	*hh*
Deceased $\geq$ 15	*N*	97	51	7.839	104.839	43.161	107	50	10.369	117.369	39.631
	B	165	98	4.297	169.297	93.703	312	93	11.412	323.412	81.588
	$\bar{B}$	1.701	1.922	0.548	1.615	2.171	2.916	1.860	1.101	2.756	2.059
	S.E.	0.202	0.385	—	—	—	0.277	0.330	—	—	—
Living $\geq$ 45	*N*	23	46	1.358	24.358	44.642	30	63	1.884	31.884	61.116
	B	57	103	1.979	58.979	101.021	74	136	4.656	78.656	131.344
	$\bar{B}$	2.478	2.239	1.457	2.421	2.263	2.467	2.159	2.471	2.467	2.149
	S.E.	0.579	0.346	—	—	—	0.392	0.252	—	—	—
Total	*N*	120	97	9.197	129.197	87.803	137	113	12.253	149.253	108.747
	B	222	201	6.276	228.276	194.724	386	229	16.068	402.068	212.932
	$\bar{B}$	1.850	2.072	0.682	1.767	2.218	2.818	2.027	1.311	2.694	2.114
	S.E.	0.198	0.260	—	—	—	0.233	0.202	—	—	—
Total	$\bar{B}_o$	1.388	1.554	—	1.325	1.664	2.170	1.561	—	2.074	1.628

*$\bar{B}_o = \bar{B}l_{15}$ where $l_{15} = 0.75$ for males and 0.77 for females. These values may be in error by about two per cent. See text.

Mean of males plus females:

	$\bar{B}$	*S.E.*	$\bar{B}_o$
Non-choreic	2.048	0.162	1.556
hh	2.162	—	1.643

The probability that this difference is due to chance is less than .001. The proportions for non-choreic males and for non-choreic females are 0.216 and 0.177, respectively. This increased marriage rate of female choreics has already been noted in Part 1 of this study. That this sex difference in proportion of married choreics may be only one factor in the difference in mean numbers of children is suggested by the fact that the mean for all married male choreics is 2.483 ± 0.232 while for all female married choreics it is 3.048 ± 0.242. These means do not differ significantly but the difference is suggestive. It is worth emphasizing here that these means are the fertilities of individuals who have survived to the age of 15 years. To obtain the mean fertility per (liveborn) newborn individual ($\bar{B}_o$) we must multiply each of the above-calculated means by the proportion of individuals of the specified category who survive to age 15, i.e. by l_{15}. Values of l_{15} are not known precisely since a direct count of deaths in these sibships between birth and age 15 is subject to some error and the division be-

tween heterozygotes and homozygotes cannot be made, while estimating l_{15} from life tables is made difficult by the wide range of birth years and geographical origins of individuals in these sibships. However, it should still be possible to estimate l_{15} for the present study from the life tables in the *United States Life Tables*, 1890, 1901, 1910, and 1901–1910 (U. S. Bureau of the Census, 1921) since these tables are based on data from ten north-eastern states, including Michigan. Since the living members of these sibships in 1940 were about 48 years of age, the l_{15} values for 1890 will be appropriate for individuals of this mean age and are an approximation for all individuals in this sample. For 1890 the l_{15} values are given only for Massachusetts (whites plus non-whites), being 0.706 for males and 0.723 for females. For 1901 and 1910 they are available for the ten states and can be further limited to whites only (native plus foreign-born). The values for these males in 1901 and 1910 are 0.780 and 0.805 respectively, while for females they are 0.807 and 0.831. It therefore seems adequate to consider that for the fertility sample composed of native and foreign-born whites, l_{15} is about 0.75 for males and 0.77 for females. The error in these estimates should not exceed two or three per cent for the general population of Michigan in 1890. Since the earliest observed age of onset was 15 years, it is believed that there is no difference in the l_{15} values of choreics, their non-choreic sibs, and the general population of Michigan. Using these values we note, in Table 6, that now, *measuring from birth*, only choreic females and *Hh* females have a $\bar{B}_o$ greater than two, while individuals in other categories have $\bar{B}_o$ values ranging from 1.325 to 1.664. Since the mean number of children required for exact replacement is two, these results indicate that only these two classes of females, on the average, are replacing themselves in the course of one complete life cycle.

Using the mean numbers of children ever born given in Table 6 we can calculate the relative fitness from ratios of means as is usually done. We use the values actually obtained in our sample since correcting to $\bar{B}_o$ involves multiplying both numerator and denominator by l_{15}. We first calculate two relative fitnesses, $W_{c \cdot n}$, defined as the ratio of the mean for choreics, of specified sex, to the mean for non-choreics of both sexes (since males and females don't differ), and $W_{H \cdot h}$, the ratio of the estimated mean for *Hh* individuals, of specified sex, to the estimated mean for *hh* individuals of both sexes. [Because of the many comparisons of relative fitness to be made, the following terminology is adopted. The subscripts of W indicate the two groups whose fitnesses are being compared, the first subscript indicating the numerator and the second the denominator. The subscripts are c (choreic), n (non-choreic sib), H (estimated Hh), h (estimated *hh* sibs), and p (general population). It should be noted at the outset that $W_{H \cdot p}$ (for the mean of males and females) is the relative fitness of genetic importance. The other relative fitnesses are interesting for other reasons or were used in obtaining the mean $W_{H \cdot p}$.] We find the following values, uncorrected for parental age distribution:

	$W_{c \cdot n} \pm$ S.E.	$W_{H \cdot h} \pm$ S.E.
Males	0.903 ± 0.120	0.817 ± 0.110
Females	1.376 ± 0.157	1.246 ± 0.142
Mean of males and females	1.140 ± 0.117	1.032 ± 0.105

The bias of these estimates is negligible compared with the standard error since the standard errors of the mean number of livebirths are about 0.1 of the mean (Cochran, 1953). The standard error of a ratio of means requires knowledge of the correlation between pairs of observations used in estimating the numerator and denominator. This correlation is assumed to be zero here since, in the present data, there is no definite mechanism operating to produce a correlation between the fertility of a choreic, taken at random, with the fertility of a non-choreic, taken at random. A positive correlation would reduce the above standard errors. In testing the significance of the W's the differences between means were used. Only $W_{c \cdot n}$ for females is significantly different from unity ($P < 0.01$). For both sexes $W_{H \cdot h}$ is about ten per cent less than that of $W_{c \cdot n}$ so that the relative fitness of heterozygotes (relative to sibs, not to the general population) is less than a simple comparison of affected with normal sibs would indicate. A fairly striking difference between males and females shown by both $W_{c \cdot n}$ and $W_{H \cdot h}$ is apparent, in each case significant at the 0.01 level.

As shown previously (Eq. 2) the definition of W should include the parental age distribution if these distributions differ in the two groups being studied. In Table 7 the distribution is given of individuals by exact age at the birth of a liveborn child. In Table 8 the distribution of these parental age groups, by five year intervals, is given, together with the parental age (P_x) for males and females in Michigan in 1935 (the earliest year for these data). Using expected values from these Michigan P_x values, the significance of departures of the observed values from the state (testing within sexes) was calculated. As is shown, only female choreics differ significantly ($P < 0.01$), having an excess of births at high ages. The parental age distributions, within sexes, cannot be shown to differ between choreics and non-choreics. Since the $W_{c \cdot n}$ and $W_{H \cdot h}$ values obtained must later be used in estimating fitness relative to the general population, the values based on female choreics must be weighted by

$$\sum_x \frac{P_{1 \cdot x}}{x} \Big/ \sum_x \frac{P_{2 \cdot x}}{x}$$

(see Eq. 2) to correct for the different parental age distribution, where subscript *1* refers to female choreics and *2* to the females of Michigan. From Table 8, using the mid-ages, 17.5, 22.5, etc., this ratio is calculated to be 0.969. The corrected values of $W_{c \cdot n}$ and $W_{H \cdot h}$ are then:

	$W_{c \cdot n}$	$W_{H \cdot h}$
Females	1.333	1.207
Mean of males and females	1.118	1.012

2. Comparison of fertility of choreic and non-choreic sibs with 1940 census fertility data

The 1940 Census (April 1, 1940) obtained information on the number of (liveborn) children ever born to white women of specified ages in Michigan. This permits direct comparisons to be made with the fertility of choreics and their non-choreic sibs who were living in Michigan on April 1, 1940. These data are presented in Table 9. It is important to note several characteristics of these data. They give mean number of livebirths per woman *surviving* to the specified age and therefore are not the desired mean nunber of livebirths per newborn individual. This latter statistic is not avail-

TABLE 7. DISTRIBUTION OF INDIVIDUALS IN TABLES 2 AND 3 BY AGE AT BIRTH OF LIVEBORN CHILD. ONLY INDIVIDUALS ALL OF WHOSE AGES AT BIRTH ARE KNOWN

Deceased $\geq$ 15 years plus living $\geq$ 45 years

x = Age at birth of child

N = Total number of individuals

B = Total number of children born to N individuals

Age x	Males		Females		Age x	Males		Females	
	Choreic	Non-choreic	Choreic	Non-choreic		Choreic	Non-choreic	Choreic	Non-choreic
15	0	0	0	1	35	7	4	10	4
16	0	0	1	1	36	4	7	10	3
17	1	2	4	2	37	7	5	6	3
18	1	0	7	5	38	3	2	4	2
19	1	4	9	7	39	3	4	7	4
20	1	2	19	10	40	6	2	9	1
21	5	3	23	5	41	3	2	2	0
22	7	5	17	9	42	2	3	2	0
23	8	2	13	12	43	2	2	3	0
24	8	9	20	9	44	3	2	3	0
25	17	6	16	7	45	1	1	1	0
26	11	10	18	7	46	2	1	0	0
27	15	3	14	7	47	2	2	0	0
28	13	6	14	10	48	3	0	1	0
29	8	10	10	9	49	0	2	0	0
30	9	5	14	4	50	1	0	0	0
31	9	8	4	11	51	2	1	0	0
32	9	4	13	4	53	0	0	0	1
33	12	6	10	6	54	1	0	0	0
34	8	7	9	3	56	1	0	0	0
					N	65	41	86	51
					B	196	132	293	147
					$\bar{x}$	31.158	31.068	27.843	26.823
					S.E.$\bar{x}$	0.539	0.649	0.401	0.515

able for any representative American population. This limitation does not, however, invalidate direct comparison between the means of the census and those of non-choreic males and non-choreic females living at the time of the census because the viability of these normal sibs should not differ appreciably from that of the general population. There are no census data for males but it is obvious that, in the absence of migration, the mean number of livebirths ever to be born to a newborn male must be very nearly equal to that of a newborn female. For ages before the end of the reproductive period the mean age differentials between spouses will make the age-specific means differ, but for ages of 40 or over this difference should be negligible. The non-choreic sibs may therefore be directly compared to the general population of females of the state in terms of fertility of surviving individuals, but this is not the

TABLE 8. COMPARISON OF OBSERVED PARENTAL AGE DISTRIBUTIONS WITH THE DISTRIBUTION IN MICHIGAN IN 1935. DATA OF TABLE 7 AND OF U. S. BUREAU OF THE CENSUS (VITAL STATISTICS—SPECIAL REPORTS)

Age Group	Number of Livebirths								Proportion of Livebirths, Michigan, 1935	
	Males				Females					
	Choreics		Non-choreics		Choreics		Non-choreics		Males	Females
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.		
15–19	3	2.35	6	1.58	21	32.82	16	16.46	.012	.112
20–24	29	35.67	21	24.02	92	92.88	45	46.60	.182	.317
25–29	64	56.25	35	37.88	72	77.06	40	38.66	.287	.263
30–34	47	44.69	30	30.10	50	50.40	28	25.28	.228	.172
35–39	24	30.18	22	20.33	37	28.42	16	14.26	.154	.097
40–44	16	16.66	11	11.22	19	10.26	1	5.15	.085	.035
45+	13	10.00	7	6.73	2	0.88	1	0.44	.051	.003
Total	196	195.80	132	131.86	293	292.72	147	146.85	.999	.999
χ^2_5*	4.53		0.58		16.22		3.07		—	—
P	>0.3		>0.9		<0.01		>0.5		—	—

* Ages 15–24 pooled for males, 40+ for females.

case for the choreics. In this latter case a bias arises as a consequence of using choreics who have reached a specified age. This produces a selection for choreics who are, on the average, less severely affected and may, therefore, differ in fertility from the mean for all choreics. One might expect that they would be more fertile because their chorea is milder. Because of this bias it was not thought worthwhile to make the additional corrections for a) the choreics who may have been selected, wholly or in part, because of having had children, and b) for non-choreics who are in fact heterozygotes and should be added to the choreics to obtain the total heterozygotes. Because of these biases it is very likely that the means for choreics are really an upper limit of the true mean for all choreics and for all heterozygotes. Since 22 individuals living in Michigan in 1940 and non-choreic at that time later developed Huntington's chorea, they are included in Table 9 in the category "Pre-choreic." The sum of choreics and "pre-choreics" should be a good approximation to the actual number of heterozygotes in 1940. Conversely, by not classifying the "pre-choreics" as non-choreics, the latter should be a good approximation to the homozygous normals.

Table 9 shows that, except for choreic females and the few "pre-choreics," individuals in almost every age group are less fertile, but usually not significantly so, than white Michigan females of the corresponding age groups. To obtain an approximation to the number of children ever born and increase the numbers of individuals in a specified category, the fertility of individuals age 40 and over, classified by whether ever married or not, was analyzed. These data are given in Table 10. It is seen that (total) male choreics are much less fertile than the state population of the same age distribution as these choreics (mean 1.867 ± 0.289; expected mean 2.836). This difference is significant at the .001 level. For total male choreics plus male pre-

choreics the difference is still significant (2.047 ± 0.317; expected 2.824) at the .02 level. The difference between observed and expected for male choreics (with or without pre-choreics) who were ever married is not significant but in each case the fertility of choreics or choreics plus pre-choreics is less. The only other significant difference for separate sexes is that between total non-choreic females and the expected (2.255 ± 0.292; expected 2.902), significant at the .02 level. However, the mean for total non-choreic males, 2.224 ± 0.373, differs from the expected 2.933, at $P = .07$, and if one makes a similar comparison for total non-choreic males age 30 or over, the difference is significant ($P = .02$), the mean again being lower than expected. In each of these cases where a significant difference is found using total individuals, the mean based on "ever married" individuals is also lower than the corresponding expected mean, but not significantly so. The mean fertilities of non-choreics do not differ significantly between sexes and therefore the fertilities of males plus females, for "ever married" and for total individuals, were calculated. For the former category the observed mean is 2.589 ± 0.249, expected 3.086, $P < .05$, and for the latter 2.240 ± 0.232, expected 2.917, $P < .01$. Since the mean number of livebirths ever born per non-choreic age 40 or more ($\bar{B}_{n\cdot 40}$) is less than that of the general population of Michigan females, ($\bar{B}_{m\cdot 40}$) it is very likely that the mean for newborn non-choreics ($\bar{B}_{n\cdot o}$) is also significantly less than the corresponding population mean ($\bar{B}_{m\cdot o}$). It is therefore inadmissible to consider the non-choreics as representative of the general population in any estimation of the relative fitness of choreics or heterozygotes. If one can assume that $\bar{B}_{n\cdot 40}/\bar{B}_{n\cdot o}$ is very near to $\bar{B}_{m\cdot 40}/\bar{B}_{m\cdot o}$ (this appears reasonable, see Reed [1959]), one can estimate the fitness ($W_{n\cdot p}$) of non-choreic sibs relative to that of the general population. We estimate this as

$$W_{n\cdot p} \doteq \frac{\bar{B}_{n\cdot 40}}{\bar{B}_{m\cdot 40}} = \frac{2.240 \pm 0.232}{2.917} = 0.768 \pm 0.080,$$

the mean value for males plus females.

3. *Estimate of the fitness of* Hh *individuals relative to the general population*

Using normal sibs and the 1940 census: If we multiply the estimates of $W_{c\cdot n}$ (corrected for parental age distribution) of the preceding section by $W_{n\cdot p}$ we will obtain estimates of fitness of choreics relative to the general population itself. We may call these estimates $W_{c\cdot p}$. To obtain an estimate of the fitness of heterozygotes relative to the general population ($W_{H\cdot p}$) we need to multiply the fitness of heterozygotes relative to non-choreic sibs ($W_{H\cdot n}$) by $W_{n\cdot p}$. The values of $W_{H\cdot n}$ obtained from Table 6, and including the previous parental age correction, are 0.863, 1.315, and 1.089 for males, females, and their mean, respectively. These estimates are:

	$W_{c\cdot p} \pm$ S.E.	$W_{H\cdot p} \pm$ S.E.
Males	0.694 ± 0.117	0.663 ± 0.107
Females	1.024 ± 0.164	0.979 ± 0.148
Mean of males and females	0.859 ± 0.129	0.821 ± 0.116

The variance of the product of two means, $\overline{xy}$, is, in part, a function of the correlation between x and y, increasing with positive correlation and vice versa. There appears

TABLE 9. FERTILITY OF CHOREICS, THEIR NON-CHOREIC SIBS, AND WHITE FEMALES OF THE GENERAL POPULATION. ALL INDIVIDUALS LIVING IN MICHIGAN ON APRIL 1, 1940. DATA AS OF APRIL 1, 1940. SEE TEXT CONCERNING BIASES IN THE MEANS OF CHOREICS RELATIVE TO OTHER MEANS

N = No. of individuals
B = No. of liveborn children (Livebirths in "1940" are counted ¼)

Age	Ever Married	Item	Males				Females				White Females, Michigan, 1910[a]
			Choreic	Pre-choreic*	Choreic + pre-choreic	Nonchoreic	Choreic	Pre-choreic*	Choreic + pre-choreic	Nonchoreic	
15–29	No	N	3	2	5	2	3	2	5	3	—‡
		B	0	0	0	0	1	0	1	0	—
		$\bar{B}$	0	0	0	0	0.333	0	0.200	0	—
	Yes	N	2	2	4	10	3	1	4	10	277,020
		B	0	1.25	1.25	6.50	2	1	3	10	342,716
		$\bar{B}$	0	0.625	0.313	0.650	0.667	1.000	0.750	1.000	1.237
		S.E.	—	—	—	0.256	—	—	—	0.258	—
	Total	N	5	4	9	12	6	3	9	13	622,240
		B	0	1.25	1.25	6.50	3	1	4	10	342,716
		$\bar{B}$	0	0.313	0.139	0.542	0.500	0.333	0.444	0.769	0.551
		S.E.	—	—	0.139	0.224	0.224	—	0.176	0.231	—
30–39	No	N	2	0	2	2	0	0	0	1	—
		B	0	0	0	0	0	0	0	0	—
		$\bar{B}$	0	—	0	0	—	—	—	0	—
	Yes	N	11	4	15	11	17	3	20	20	307,140
		B	21	5	26	16	50	5	55	35	668,540
		$\bar{B}$	1.909	1.250	1.733	1.455	2.941	1.667	2.750	1.750	2.177
		S.E.	0.368	—	0.316	0.312	0.539	—	0.481	0.428	—
	Total	N	13	4	17	13	17	3	20	21	343,380
		B	21	5	26	16	50	5	55	35	668,540
		$\bar{B}$	1.615	1.250	1.529	1.231	2.941	1.667	2.750	1.667	1.947
		S.E.	0.368	—	0.311	0.303	0.539	—	0.481	0.416	—
40–49	No	N	5	0	5	1	2	0	2	2	—
		B	1	0	1	0	0	0	0	0	—
		$\bar{B}$	0.200	—	0.200	0	0	—	0	0	—

40–49	Yes	*N*	17	3	20	10	29	5	34	15	269,180
		B	37	14.25	51.25	27	90	22	112	33	766,772
		$\bar{B}$	2.177	4.75	2.563	2.700	3.103	4.400	3.294	2.200	2.849
		S.E.	0.472	2.529	0.552	0.817	0.531	1.123	0.482	0.536	—
	Total	*N*	22	3	25	11	31	5	36	17	289,720
		B	38	14.25	52.25	27	90	22	112	33	766,772
		$\bar{B}$	1.727	4.750	2.090	2.455	2.903	4.400	3.111	1.941	2.647
		S.E.	0.407	2.529	0.481	0.779	0.515	1.123	0.473	0.504	—
50+	No	*N*	6	0	6	5	4	0	4	6	—
		B	0	0	0	0	0	0	0	0	—
		$\bar{B}$	0	—	0	0	0	—	0	0	—
		S.E.	0	—	0	—	—	—	—	0	—
	Yes	*N*	17	0	17	33	42	0	42	32	373,520
		B	46	0	46	82	135	0	135	91	1,186,644
		$\bar{B}$	2.706	—	2.706	2.485	3.214	—	3.214	2.844	3.177
		S.E.	0.452	—	0.452	0.469	0.475	—	0.475	0.376	—
	Total	*N*	23	0	23	38	46	0	46	38	393,420
		B	46	0	46	82	135	0	135	91	1,186,644
		$\bar{B}$	2.000	—	2.000	2.158	2.935	—	2.935	2.395	3.016
		S.E.	0.417	—	0.417	0.429	0.454	—	0.454	0.359	—
Total‡‡	No	*N*	16	2	18	10	9	2	11	12	—
		B	1	0	1	0	1	0	1	0	—
		$\bar{B}$	0.063	0	0.056	0	0.111	0	0.091	0	—
		S.E.	0.063	—	0.056	0	0.111	—	0.091	0	—
	Yes	*N*	47	9	56	64	91	9	100	77	1,226,860
		B	104	20.50	124.50	131.50	277	28	305	169	2,964,672
		$\bar{B}$	2.213	2.278	2.223	2.055	3.044	3.111	3.050	2.195	2.416
		S.E.	0.260	0.999	0.266	0.292	0.296	0.824	0.278	0.229	—
	Total	*N*	63	11	74	74	100	11	111	89	1,648,760
		B	105	20.50	125.50	131.50	278	28	306	169	2,964,672
		$\bar{B}$	1.667	1.864	1.696	1.777	2.780	2.545	2.757	1.899	1.793
		S.E.	0.227	0.855	0.299	0.265	0.282	0.767	0.264	0.214	—

* Non-choreic on April 1, 1940, choreic later.
** U. S. Census. Data on total white women, except ever married and not reporting on children.
‡ Single women were not asked about children.
‡‡ Means for total choreics and total non-choreic sibs are not comparable with the total state population since the age distributions are very different.

TABLE 10. FERTILITY OF CHOREICS AND THEIR NON-CHOREIC SIBS AND THE EXPECTED FERTILITY ($\bar{B}_m$) OF WHITE FEMALES IN MICHIGAN WITH SIMILAR AGE DISTRIBUTION. ONLY INDIVIDUALS AGE 40 YEARS OR OVER. DATA AND SYMBOLS OF TABLE 9

Ever Married	Item	Males				Females			
		Choreic	Pre-choreic	Choreic + pre-choreic	Non-choreic	Choreic	Pre-choreic	Choreic + pre-choreic	Non-choreic
No	*N*	11	0	11	6	6	0	6	8
	B	1	0	1	0	0	0	0	0
	$\bar{B}$	0.091	—	0.091	0	0	—	0	0
Yes	*N*	34	3	37	43	71	5	76	47
	B	83	14.25	97.25	109	225	22	247	124
	$\bar{B}$	2.441	4.750	2.628	2.535	3.169	4.400	3.250	2.638
	S.E.	0.325	2.528	0.359	0.403	0.353	1.123	0.338	0.308
	$\bar{B}_m$	3.013	2.849	3.000	3.101	3.043	2.849	3.030	3.072
Total	*N*	45	3	48	49	77	5	82	55
	B	84	14.25	98.25	109	225	22	247	124
	$\bar{B}$	1.867*	4.750	2.047*	2.224	2.922	4.400	3.012	2.255*
	S.E.	0.289	2.528	0.317	0.373	0.339	1.123	0.327	0.292
	$\bar{B}_m$	2.836	2.647	2.824	2.933	2.867	2.647	2.854	2.902

$\bar{B}$ for non-choreic males plus non-choreic females:

	Observed	Expected	*P*
Ever married	2.589 ± 0.249	3.086	< .05
Total	2.240 ± 0.232	2.917	< .01

* = different from expected at 5 per cent level of significance.

to be no obvious cause for positive correlation between $W_{c\cdot n}$ and $W_{n\cdot p}$ or between $W_{H\cdot n}$ and $W_{n\cdot p}$. There is some reason, however, to believe that any correlation might be negative, since the mean fertility of non-choreics occurs in the denominator of $W_{c\cdot n}$ and $W_{H\cdot n}$ and in the numerator of $W_{n\cdot p}$. If this were so, the above standard errors, which assume no correlation, would be reduced slightly. Using the standard errors as calculated (which are based on the standard errors for $W_{c\cdot n}$ and $W_{H\cdot h}$, believed to be slightly too large) $W_{c\cdot p}$ and $W_{H\cdot p}$ for males are seen to be significantly different from unity, indicating that male choreics and male *Hh* are less fit than the general population. Females, and the mean of males and females, however, do not differ significantly. However, since the value for the mean $W_{H\cdot p}$, 0.821, (which is *the* relative fitness of genetic importance) is based half on the fitness of male *Hh* which do differ significantly ($P < .01$) from unity, it really is different from unity. This comparison therefore indicates that heterozygotes are less fit genetically than the general population, having a relative fitness of about 0.82 of normal.

Using estimate of $\bar{B}_o$ obtained from Cohort Fertility: The preceding estimate of the mean $W_{H\cdot p}$ was partly based on the mean fertilities of living normal sibs and the 1940 census fertility data because a direct comparison of choreics or heterozygotes with census data is not valid. An estimate of $W_{H\cdot p}$ not dependent on the non-choreic sibs is desirable, however. The fertility data assembled by Whelpton (1954) can be used for such an estimate. Using all available United States census data on fertility

and mortality, Whelpton (1954) has calculated, among other quantities, the mean number of liveborn children ever born ($\bar{B}_s$) to U. S. native-born white women, of certain specified cohorts, who *survive* to specified age x. ("Cohort" here is all of the above-specified liveborn females who are born in a particular year, say 1890). The mean number of liveborn children per original member of the cohort ($\bar{B}_o$) is not given here, nor is it available elsewhere. $\bar{B}_s$ and $\bar{B}_o$ differ appreciably for high x, $\bar{B}_s$ exceeding $\bar{B}_o$, because $\bar{B}_s$ is based only on women who survive to, say, 40 years, neglecting those who die below this age. If one knew the age-specific cumulative fertility of women dying at age x, it would be possible to calculate $\bar{B}_o$ exactly. This fertility is unknown but, as a first approximation, one can assume it is the same as that of women living at age x. It is possible to think of reasons why it should be lower; it is also possible to find reasons why it may be higher. Our interest is in the cohort of 1890 since this approximates the present data well. By multiplying the number of women dying at age x (obtained by subtraction from Table C, Whelpton, 1954) by the cumulative fertility at age x (mean value for age x in Table A, *ibid.*), and summing these products from birth to age 47, the number of births to women dying before age 47, B_d, is obtained. B_d plus the total births from women reaching age 47 or over gives the total births from the original cohort. For the cohort of 1890 these data are not given before age 30 so that it is necessary to estimate these early births from data on the cohort of 1900. (The authors are indebted to P. K. Whelpton, Scripps Foundation for Research in Population Problems, for suggesting this general procedure. The calculations are our own.) If the cohort of 1890 numbered 10,000 liveborn females, it may be calculated that 536 children are born to women dying under 30, 1,538 to women dying between 30 and 46 inclusive, and 17,753 to women surviving to 47 or over. The total of 19,827 children is equivalent to 1.9827 livebirths ever born per original member of the cohort. The accuracy of this estimate is somewhat uncertain but it is clear that the true value is appreciably greater than the minimum estimate of 1.7753, the value which would result if women dying under 47 had no children. An absolute maximum, clearly too high, is given by assuming that all women reaching age 15 survive through age 47. This maximum is 2.340 children ever born. The estimate of 1.9827 is below replacement value, but this is believed to have been characteristic for several native white American cohorts of this period (P. K. Whelpton, personal communication). Since this estimate is for native-born white women of all the United States, we may approximate the corresponding value for Michigan white (native-born plus foreign-born) women by multiplying 1.9827 by the ratio, for women age 45–49 years in 1940, of a) number of livebirths ever born per Michigan white women to b) number of livebirths ever born per U. S. native white woman. This ratio is $2.730/2.602 = 1.049$. The estimate for a Michigan cohort of 1890 is then 2.080, slightly over replacement. This estimate of $W_{H\cdot p}$ requires an estimate of $\bar{B}_o$ for all Hh individuals. From Table 6 and from the weighting factor of 0.969 to correct for the parental age distribution of female choreics, this is $[1/2]\ [1.325 + 0.969 \times 2.074] = 1.668$. The mean $W_{H\cdot p}$ is then $1.668/2.080 = 0.802$.

Using the estimate of $\bar{B}_o$ for the general population derived by Reed (1959): Reed (1959) calculated $\bar{B}_o$ for the normal sibs of sporadic (parents normal) propositi of individuals affected with multiple neurofibromatosis, using published and unpublished

TABLE 11. PROPORTION OF LIVEBORN CHILDREN OF CHOREICS AND OF NON-CHOREIC SIBS DYING UNDER FIVE YEARS OF AGE. ONLY CHILDREN BORN FIVE OR MORE YEARS BEFORE THE TIME OF LAST INVESTIGATION TO WHITE, MARRIED PARENTS

	Male Parent		Female Parent	
	Choreic	Non-choreic	Choreic	Non-choreic
Total number of children	314	266	529	360
Number dying under 5 years	15	10	40	17
Proportion dying	0.048 ± 0.012	0.038 ± 0.012	0.076 ± 0.011	0.047 ± 0.011

Proportion dying under five, non-choreic parents, males plus females: 0.043 ± 0.008

data of Crowe *et al.* (1956). These propositi were ascertained in Michigan between 1934 and 1953. Because their parents and ancestors were normal these sibs are believed to be representative of the general population. A comparison of their completed fertility with data of the 1950 census confirms this belief; no significant differences were found. For 107 such individuals who had lived to age 40 years or over, or who died at any age, $\bar{B}_o$ was found to be 2.037 ± 0.240 livebirths ever born. Using this value, the mean $W_{H\cdot p}$ is estimated to be 1.668/2.037 = 0.819. The relatively large standard error of $\bar{B}_o$ makes this estimate less reliable than the preceding two but it is useful as a check.

The "best" estimate of $W_{H\cdot p}$: Since the estimates vary only from 0.802 to 0.821, the range being less than the smallest standard error, the question of which is "best" is rather academic. It seems reasonable to use the mean of the three estimates as the "best" estimate. This is 0.81, to two decimals.

4. *Infant mortality among the children of choreics and non-choreic sibs*

Panse (1942) reported an increase in infant mortality among children of choreics, (28.64 ± 1.8 per cent dying in the first 10 years of life for children born 1880–1899) relative to that in children of non-choreic sibs of choreics (22.64 ± 2.08 per cent in the first 10 years). He does not distinguish the sex of the choreic parent. This possible mode of selection was investigated in the present study by considering mortality between birth (livebirth) and the fifth birthday. These data are presented in Table 11. The proportion of deaths among children of male non-choreics does not differ significantly from that of female non-choreics so we may pool these groups to obtain an estimate for non-choreics, in general, of the proportion of liveborn children dying before their fifth birthday, 0.043 ± 0.008. The proportion for male choreics does not differ significantly, being 0.048 ± 0.012, but that of females does, being 0.076 ± 0.011 ($\chi_1^2 = 4.958$, $P = .025$). If this difference between male and female choreics is real it is not surprising since the mother is probably more important to an infant's survival than is the father. These data indicate that the real fitness of married female choreics should be decreased by about 0.033 and that of all choreics by about 0.015.

MUTATION

Almost all rare dominant traits in man with high penetrance, when intensively studied, give examples of mutation. Dentinogenesis imperfecta (Witkop, 1957) is

perhaps the only such trait which, after a large-scale investigation, yielded no evidence for mutation. It is therefore appropriate to look for mutation in Huntington's chorea even though, because of its high relative fitness and late onset, good examples are expected to be rare.

The possibility of very late onset of the symptoms of chorea in individuals who are *Hh* is a serious handicap to any attempt to demonstrate mutation. In fact, we consider it impossible, with the present diagnostic facilities, to pick out individual families in which mutation has occurred. At the same time, if one studies a large number of choreic families it may be possible to demonstrate with considerable reliability that mutation is occurring in some of the families. The latest onset of choreiform movements among 204 choreic individuals in the present study was 65 years, this onset age being found in two individuals. The next highest age was 54 years, again occurring in two individuals. These ages indicate the difficulty of proving mutation in specific cases in the face of the alternative of late onset in a parent. An instructive example of this difficulty can be given. Through correspondence we learned of a patient, male, age 44 years, with Huntington's chorea whose parents were reported normal at ages 77 years (father) and 68 years (mother). The patient's wife, the family physician, and the superintendent of the nearby mental institution to which the patient was committed, all stated that the parents and all other relatives were normal. Dr. J. H. Chandler, then of this Department, visited the family (Kindred #4655) (which lives outside of Michigan), and made neurological examinations on the patient, his brother (age 41 years), and both parents. Typical Huntington's chorea was found in both the patient and his brother. Definite, but mild, symptoms of this disease were also observed in the father (e.g. flexion-extension movements at fingers and wrists, jerking movements of shoulders, torsions of the trunk, fleeting universal flexion of lower extremities). The mother appeared entirely normal. Since the family (whose members were intelligent) did not recognize any abnormality in the father, his age at onset is unknown. The important point is that without careful examination this family would have been thought to offer good evidence for mutation, considering the ages of the parents. Other workers have also noted very late onset, 70 years or later (e.g., Entres, 1921; Bell, 1934; Brothers, 1949).

Although we do not believe that specific instances of mutation in Huntington's chorea can be demonstrated, we have recorded, as a matter of interest, the number of instances where a) both parents of a *single* case of Huntington's chorea reached the age of 60 or over and b) were reported to be normal. The kindreds containing parents meeting both requirements are therefore possible examples of mutation. This procedure, however, cannot be used to obtain an estimate of the mutation rate because, obviously, mutation can occur in young parents as well as old and these young parents are excluded. In 196 apparently separate kindreds (groups of biologically related individuals) of Huntington's chorea, one or more of whose choreic members (medically diagnosed) lived in Michigan at some time, there was sufficient information (although usually second-hand) to classify the parents. In eight of the 196 kindreds both were at least 60 and had only one choreic child. Only four of these eight kindreds could be thoroughly investigated and of these four, the father in one was found to have committed suicide at age 62, raising the question of his being in the early stages

of the disease. In the kindred presenting the strongest case for mutation (Kindred #4455) the propositus, who was the only case of Huntington's chorea in the kindred, died at age 38 years. Autopsy findings, including brain sections (reviewed by Dr. J. H. Chandler), were typical of the disease. Four sibs, ranging in age from twenty to forty-four years, were reported to be normal. The parents, both age 65 years, were visited by a trained fieldworker, familiar with early and late stages of the disease, and were found to be normal. The parents had no knowledge of the disease in their own ancestors. These facts definitely suggest that mutation has occurred within the last several generations of this kindred but, in view of the fact that this kindred is one of 196 kindreds, we may have merely selected for late onset in the heterozygous ancestors of the patient, all of whom died before showing symptoms of the disease. It is therefore not justifiable to conclude that this example demonstrates mutation. The possibility of diagnostic error, when there is only one choreic in a kindred, is further reason to be cautious.

If, as seems probable, mutation from the normal allele to an allele causing Huntington's chorea does occur, it is obvious that an upper limit of the frequency of such mutation, μ [mutations/locus (loci, *if* there are several producing the phenotype of Huntington's chorea)/generation], can be found. If we classify each individual in Michigan on April 1, 1940 who was known to be *Hh*, (either being choreic at that time or developing chorea at a later date) according to whether or not one of his parents appeared to be *Hh*, being classified *Hh* because of being choreic or, if not choreic, having two or more choreic children and/or a collateral or antecedent choreic relative, we find the following: Of 231 *Hh* individuals known, 206 had an *Hh* parent, 7 were not known to have an *Hh* parent, and 18 could not be classified, usually because they had no known relatives in Michigan. A maximum estimate of the proportion of *Hh* individuals lacking an *Hh* parent, p, is clearly given by $25/231 = 0.108$. The frequency of *Hh* individuals in Michigan, f, was estimated in the first part of this study as 1.01×10^{-4}. Therefore, whether or not the population is in equilibrium with respect to origin and loss of *H* genes, $\mu_{max} = pf/2 = 5.4 \times 10^{-6}$. This is clearly a maximum estimate and probably is several times too high.

No useful estimate of the minimum mutation rate can be derived from the present data. The fact that of 231 *Hh* individuals only 7 are reported to have had normal parents (neither known to be *Hh*) would seem reasonable even if we knew that there were no mutation. If, in fact, there were no mutation this small proportion of *Hh* parents might be expected not to show chorea because of failure to reach the age of onset. Examination of the ages at death or time of last investigation of these seven pairs of parents is not very informative. These ages are, giving the father's ages first: (75, 21), (70, 25), (63, 40), (48, 71), (80, 55), (62, 70), and (69, 87). The P_x and C_x statistics, previously derived from the age of onset distribution, if reliable at advanced ages, could be used to calculate the probability that both members of some of these pairs of parents are *hh* and, therefore, demonstrate mutation. Unfortunately, such reliability cannot be assumed, but, even if it existed, the problem of sampling error would be very difficult. For these reasons no estimate of the minimum mutation rate is attempted.

If the population is in genetic equilibrium with respect to Huntington's chorea,

which is quite possibly not the case, we may estimate μ from $\mu = (1 - W)f/2$, where W is the relative fitness estimated previously, 0.81. Using this value of W, μ is estimated to be 9.6×10^{-6}, almost twice the previously calculated μ_{max}. Since the 95 per cent confidence interval of μ_{max}, based on the estimation of p, does not include 9.6×10^{-6}, and the true value of μ is believed to be at least several times less than μ_{max}, there is reasonably good evidence that either the estimated value of W is in error or that the assumption of genetic equilibrium is incorrect. f is used in both estimates and is therefore not implicated here. It is quite possible, of course, as a result of sampling error or some unknown bias, that W is in error. For example, if the true μ is 2×10^{-6}, the observed f is correct, and genetic equilibrium exists, then W should be 0.96. On the other hand, the assumption of genetic equilibrium for Huntington's chorea seems inherently dubious. The possibility of recent change in the social and psychological characteristics of our society, which can affect the reproduction of *Hh* individuals, is obvious. Although no decision between these two alternatives, or a combination of them, can be made, lack of genetic equilibrium seems most likely to be the explanation. It may be noted in passing that these data demonstrate the uncertainty of indirect estimates of μ when W is near unity since small changes in W may produce large changes in $1 - W$.

In summary, several examples of possible mutation were observed but, because of the possibility of late onset of chorea definite instances of mutation could not be demonstrated. A direct *maximum* estimate of the mutation rate was calculated: 5.4×10^{-6}. If mutation occurs it probably does so at an appreciably lower rate than this.

DISCUSSION

The general difficulties in estimating relative fitness for specific traits, as well as the additional difficulties in the particular case of Huntington's chorea, have been discussed at some length by Reed (1959). The present study has shown how these difficulties are made acute when the relative fitness is high, around 0.8 (mean $W_{H \cdot p}$) in this case, instead of being under 0.5 as is the situation for most genetic traits for which estimates of W have been obtained. Biases which are completely negligible when W is near zero may be very important when W approaches one, thus making the definition of W more difficult than has usually been the case. Examples of such biases are a) age differences between affected and normal sibs (since fertility is age-dependent) when incompleted fertilities are used, b) fertility differences between the normal sibs of the affected and the fertility of the general population, and c) differences in the parental age distribution ($P_{i \cdot x}$ as defined on page 108). The definition of W proposed in equations (1) and (2) is designed to eliminate a) and c). Bias a) is obvious but bias b) and bias c) apparently have not been recognized previous to the findings of this study. The demonstration that b) occurs made it necessary to make all estimates with reference to the general population of Michigan ($W_{H \cdot p}$) and not with respect to the unaffected sibs ($W_{c \cdot n}$) as is usually done. The increasingly available age-specific census data on number of children ever born makes this procedure practical in a number of countries.

Bias in W as a consequence of its usual definition of the ratio of mean numbers of

children uncorrected for parental age distributions, has not usually been recognized. Reed (1959) discussed this and derived equations (1) and (2) to correct for it. The finding that the parental age distribution of female choreics differs significantly from the females of Michigan provided an example of this type of bias.

The estimate of 0.81 for Huntington's chorea and the estimate of 0.78 for multiple polyposis of the colon, (Reed and Neel, 1955), are among the highest relative fitnesses determined for rare dominant or sex-linked recessive traits, clearly a consequence of the late age of onset in these traits. It is probable that there are rare genetic traits, for example dentinogenesis imperfecta, with higher *W*'s but such estimates do not seem to have been made.

It is not known why the normal non-choreic sibs were less fertile than the females of Michigan. Reed and Chandler (1958) found no significant differences between these sibs and the general population with regard to occupation and marital status and the general impression gained was that there were no remarkable differences. An obvious possibility, for which we have no evidence, is that in kindreds having a number of affected persons some of the normal members, or their spouses, limit reproduction because of the fear of developing the disease or of transmitting it to their children. Since the fertility of "ever married" non-choreics as well as total non-choreics is decreased, another possibility is that non-choreics marry later, on the average, than the general population. Unfortunately, we do not have data on age at marriage for non-choreics. Although the mechanism causing this reduction in fertility is unknown, it is an interesting example of "gene action" in man. No other published examples of decreased fertility of normal sibs of individuals with genetic diseases are known to the authors. Data on the fertility of non-choreic sibs given by Panse (1942), S. C. Reed and Palm (1951), and Kishimoto *et al.* (in press) do not permit a comparison with census fertility data since distributions by age and year are not presented. It is worth noting that a tabulation by Reed (1959) of the original data of Crowe, Schull, and Neel (1956) on neurofibromatosis, obtained in a partial survey in the state of Michigan, revealed no indication of a difference in fertility between normal sibs of sporadic propositi and the female population of Michigan. For the normal sibs of familial (one parent affected) propositi, however, the mean fertility was appreciably, but not significantly, lower than the females of Michigan. Since most normal sibs of choreics have an affected parent, the question seems raised whether the presence of an affected parent, or other ancestor, depresses the fertility of unaffected individuals. Further data are clearly required.

The reduced fitness of *Hh* individuals (i.e., the mean for males and females) may have several explanations. The proportion of choreics who marry could not be shown by Reed and Chandler (1958) to differ significantly from the general population but the proportion may, in fact, be lower because male choreics appear to have a lower marriage rate. A more definite factor is the termination of reproduction because the disease is sufficiently advanced to require either institutionalization or other segregation. To estimate the magnitude of this factor, use can be made of the "Relative Reproductive Span" (*RRS*) (Reed and Neel, 1955), assuming (for this calculation only) that the only cause of reduced fitness is institutionalization of some choreics before the end of their potential reproductive period. The age at time of first insti-

tutionalization from Table 16 of Reed and Chandler (1958) was used, equating this age to the termination of actual reproduction, and the distribution of parental ages for Michigan in 1935 (Reed and Neel, 1955) was also used. Under these assumptions W is about 0.93. If, as is probable, fertility is reduced before this age, W would be less. Since the estimate of 0.81 doesn't differ significantly from 0.93 this factor alone might explain all of the reduction in fitness, but this does not seem likely.

The significant difference in fitness observed between male and female choreics, and also *Hh* individuals, is similar to that noted by Panse (1942). He found (see his Table 20) that male choreics had a mean of 3.13 children while female choreics had a mean of 3.56, the ratio of these means being 0.88. The significance of this difference cannot be determined from his data but it probably does not quite reach significance. Kishimoto *et al.* (in press), using data from Japanese pedigrees, report a mean fertility of 3.53 for male choreics and 3.88 for female choreics. These differences are very probably not significant. Other data on fertility, by sex, of choreics do not appear to be published and, in fact, the only other dominant trait similarly analyzed seems to be neurofibromatosis (Crowe, Schull, and Neel, 1956). [The calculation of Vogel (1957) on retinoblastoma, which found no difference between males and females in fertility, employed unilaterally affected adults of varying ages.] Using the method advocated by Krooth (1955), the relative fitness of affected males was found to be 0.413, while that of affected females was 0.748. The significance of these differences was not determined. Calculations by Reed (1959), using the original data of Crowe *et al.*, of the mean completed fertility gave values of 0.451 ± 0.208 livebirths for affected males and 1.292 ± 0.383 livebirths for affected females. These means do not quite differ significantly ($P = .07$) but the difference is suggestive. Although, except for the present study, we lack strong evidence for the reality of these differences, it is interesting that in each study the males appear less fertile. In each case (except for the data of Kishimoto *et al.*, which do not mention the marriage frequency) this seems to be in part, a result of a lower marriage rate among affected males. A reasonable explanation for much, if not all, of the decrease in the proportion of male choreics who marry, relative to that of female choreics, is given by the higher age at time of first marriage of males compared to females. If m_x is the proportion, out of all first marriages in the general population, which occur at age x, and P_x, as defined previously, is the probability that an *Hh* individual will develop Huntington's chorea by age x, then the probability that an *Hh* person marries at age x is approximately proportional to $m_x(1 - P_x)$. $M = \sum_x m_x(1 - P_x)$ should be approximately proportional to the probability that an *Hh* person ever marries. Data to estimate m_x are not available for Michigan before 1950, but data for the neighboring state of Wisconsin for 1922 (when many of the choreics in our study were marrying) should be suitable. $M_{\sigma^{\prime}}$ is found to be 0.752 and M_{φ} is 0.834; their ratio is 0.902. The mean age at marriage is 28.18 years for males and 24.62 years for females; these are slightly high because the data are for all marriages, not first marriages. (Mean age at first marriage for Michigan in 1950 was 24.88 years for males and 21.89 years for females.) From Tables 4 and 5 the observed proportions of male choreics ever marrying is seen to be 0.742, for female choreics 0.920, giving a ratio of 0.807. Thus, the fact that, on the average, a male *Hh* individual is less likely to be non-choreic at the time of his

(actual or potential) marriage than is a female *Hh* individual, seems capable of explaining much of the observed difference. This mechanism is a good example of interrelationship of genetic and social factors in determining fertility. Other possible explanations might be considered but they appear to lack reasonable support. Another example of such interrelationships is the increased mortality found among the children of female choreics but not of male choreics. This increased mortality decreases the relative fitness for all choreics by only about 1.5 per cent from the values estimated on the assumption of no difference in mortality, but is a good example of selection operating at a stage of the life cycle not usually examined.

There appear to have been only three studies, in addition to the present one, in which numerical estimates of the relative fertility of choreics have been obtained. Of these, only Panse (1942) has fertility data from a complete, or nearly complete, survey of a large population, in this case the Rhineland of Germany. In his Table 20, Panse presents data on the number of children born to 457 choreics and 505 non-choreic sibs, all age 30 or over and apparently ascertained through their parents. One can calculate from his data that the mean number of children of choreics is 3.344, that of non-choreics 2.837, giving an estimate of $W_{c \cdot n}$ of 1.18. This is an excellent agreement with the estimate of 1.14 found in the present study (uncorrected for parental age). There is a bias which will increase his value of $W_{c \cdot n}$ although it probably is not of major importance. This bias is a consequence of his minimum age of 30 since, for the age range 30–45, say, choreics will have a higher mean age than their non-choreic sibs because the probability of ascertaining choreics increases with age but the probability for ascertainment of non-choreic sibs does not. This greater mean age in turn produces a greater mean fertility. Panse did not estimate $W_{H \cdot h}$ but it seems probable that such an estimate again would not differ appreciably from our own. He did not compare the choreics' fertility with census fertility data but instead had his own data on 219 normal, more distant, relatives (often first cousins) of choreics, age 30 and over, whose fertility was known. But since the mean fertility of these relatives is 1.973 children, it is difficult to believe that they adequately represent the general population. In fact, since they are close relatives of choreics, their lower fertility raises the question of whether the same mechanism is responsible for their lowered fertility as for that of the normal sibs in the present study. It is therefore not known whether the non-choreic sibs differ from the general population of the Rhineland.

S. C. Reed and Palm (1951) presented data on the fertility of an unstated number of choreics and their non-choreic sibs in the state of Minnesota, U. S. A. They reported a mean number of 6.07 $\pm$ 0.9 children born to choreics and 3.33 $\pm$ 0.5 born to non-choreic sibs. These figures are from the data of Palm (1953) who indicates that they are based on 29 choreic and 49 non-choreic individuals, respectively, who were married and had at least one livebirth. Palm (1953) also reports a significant (at the five per cent level) difference in the mean fertility of 34 choreics and 60 non-choreics unselected for having had children, the respective means being 5.15 $\pm$ 0.87 and 2.72 $\pm$ 0.46. The age distributions are not reported nor is the method of ascertaining these individuals. It is not stated that choreic parents who were ascertained only through their children were excluded from the calculations. The ratio 5.15/2.72 =

1.89 differs markedly from the $W_{c \cdot n}$ ratios found by Panse and in the present study. It is possible that, among other factors, such as age difference between choreics and non-choreics, the fact that they ascertained only a small fraction of the Huntington's chorea kindreds in Minnesota accounts for their high value for $W_{c \cdot n}$. When ascertainment is quite incomplete the probability of ascertaining a kindred is approximately proportional to the number of choreics (or the number known to official sources). As a result there is selection for large kindreds, and consequently, fertile choreics. S. C. Reed and Palm (1951) also discuss a single large kindred, finding that the total number of descendants of a choreic man is four times that of his normal brother. It is not demonstrated that this kindred is representative of choreic kindreds in Minnesota and the mode of ascertainment of the kindred does not exclude bias for large size and hence, fertile choreics. Their data clearly show that in some kindreds choreics are very fertile, but it does not follow that the mean fertility for the state is as high as their results suggest. In our data, for example, there are many kindreds whose choreics are indeed very fertile; there are also others in which choreics are conspicuously less fertile than their normal sibs, usually because of early onset, institutionalization, and death. S. C. Reed and Palm (1951) and Palm (1953) also report that a tabulation of published pedigrees also shows greater fertility (but not significantly so) for choreics than for non-choreic sibs. Since, except for those of Panse (1942), none of these pedigrees are from complete surveys, the above criticisms concerning age distribution, method of ascertainment, and bias for large size also apply here. Because of these biases we do not believe that the data reported by S. C. Reed and by Palm are adequate for estimating the relative fitness of choreics, not to mention that of heterozygotes.

The third study is that of Kishimoto *et al.* (in press) and Kishimoto (personal communication) in Japan, the only data not on Caucasian populations. Fifty-five Japanese kindreds, published and unpublished, were used for the fertility calculations. The mean age of the choreics, living and dead, was about 51 years. No restrictions as to age or ascertainment are mentioned. They found that 58 male choreics had 3.53 children, 56 female choreics had 3.88 children, and 23 sibs (from Aichi Prefecture only, mean age 51 years) had 5.71 children. Using these figures they estimate relative fitness for choreics as 0.649. However, Kishimoto (personal communication) reports that if one compares only choreics and non-choreic sibs studied in his survey of Aichi Prefecture (numbers and ages not specified), the relative fertility of choreics is 0.85. An alternative estimate of the fertility of the general population of Japan can be obtained from the 10 per cent sample tabulation of the 1950 census. The mean number of children ever born per Japanese woman age 50–54 years in 1950 was 4.719. Valid comparisons are not possible since the age and year distributions of choreics are not given and their data are too heterogeneous and subject to bias for large size to yield a valid estimate of W. It does appear, however, that in Japan choreics (and therefore heterozygotes) are also less fertile than the general population, especially considering that, because of biases, the reported choreic fertilities are probably too high. It seems quite possible that the relative fitness of heterozygotes in Japan may be less than that in populations of European ancestry and it is tempting to correlate this with the higher fertility of the general population in Japan. Kishimoto

et al. also reported a frequency estimate for Huntington's chorea, in Aichi Prefecture, of 3.8×10^{-6}, about one-tenth that found in the U. S. A. and in Europe. For a fixed mutation rate and genetic equilibrium, lower relative fitness should produce a lower frequency. Whether this relationship explains these differences between Japan and other countries must remain speculative.

Estimation of mutation rates in dominant traits with late onset like Huntington's chorea and multiple polyposis of the colon (Reed and Neel, 1955) is of necessity indirect and inferential. Nevertheless we believe that the procedure followed in the present study provides a firm upper limit of the mutation rate. We consider it almost certain that mutation does occur since Huntington's chorea is now, and in the past has been, a rare disease, implying a relative fitness less than one. Recurrent mutation seems to be the only way in which the present frequencies can be maintained. The inability to specify a minimum rate of mutation was a consequence of the definite possibility of non-penetrance as an alternative to mutation.

Kishimoto *et al.* (in press) estimate a mutation rate for Huntington's chorea by the indirect method, using their estimate of W and frequency. Their estimate is 6.7×10^{-7}. As mentioned above, this estimate of W is subject to several biases and the calculation is not made in terms of heterozygotes, but this estimate may give the order of magnitude. Several authors, e.g. Panse (1942), mention the possibility of mutation but make no estimates. Our estimate of 5.4×10^{-6} is one of the lowest human mutation estimates made, in keeping with the high estimate for relative fertility of about 0.8. It seems unlikely that accurate or even minimum estimates will be possible until diagnostic techniques improve.

SUMMARY

This paper is the second of three papers based on data from a survey of all known cases of Huntington's chorea (H.c.) in the lower peninsula of the state of Michigan, U. S. A. The first paper (Reed and Chandler, 1958) was concerned with certain demographic and genetic aspects while the third will consider clinical features of the disease.

For the study of natural selection all sibships containing one or more individuals with H.c. living in the lower peninsula of Michigan on April 1, 1940 were considered. From these sibships choreic individuals and non-choreic sibs meeting the following requirements were chosen: 1) classification as "choreic" or "non-choreic" is adequate, 2) ascertainment was independent of fertility, 3) the individual was living at age 45 years or over, or was deceased at age 15 years or over, and 4) one parent of these sibships was very probably heterozygous for the gene for H.c. Two hundred fifty-seven choreics and 210 non-choreic sibs met these requirements. Requirement 3) ensured that only essentially completed fertilities were being measured. Requirement 4) was necessary for the estimation from the age of onset distribution, of the proportion of non-choreic sibs who were in fact unrecognized heterozygotes for the gene for H.c. This proportion was found to be about 10 per cent.

The relative fitness of choreics, when compared to their non-choreic sibs, is about 1.12 ± 0.12, while the relative fitness of heterozygotes (i.e., overt choreics plus individuals heterozygous for the gene but thus far without chorea), compared to homozy-

gous normal sibs, is about 1.01 ± 0.11. The fertility of male choreics (or heterozygotes) was significantly less than that of female choreics (or heterozygotes).

A significant difference between the fertility of the non-choreic sibs and the general population was found. This comparison utilized non-choreic sibs living on April 1, 1940, who were 40 or more years old, and fertility data of the 1940 U. S. census on females having the same age distribution. The relative fertility of the non-choreic sibs is about 0.77 ± 0.08 of the latter. The estimate of the relative fitness of individuals heterozygous for the H.c. gene, *compared to the general population*, is about 0.81.

For the study of mutation, 196 kindreds (groups of biologically related individuals) were available. No specific instances of mutation in H.c. could be demonstrated because non-penetrance could not be definitely excluded. It is noteworthy, however, that in only eight of these kindreds were both parents of a single case of H.c. 1) 60 years of age, or older, and 2) normal. Because mutation occurs in almost all well-studied rare dominant traits in man and also because the relative fitness of H.c. heterozygotes is less than that of the general population, we believe that mutation occurs in H.c. From consideration of the age and diagnostic status of the parents of the heterozygous individuals living in Michigan on April 1, 1940, a direct estimate of the *upper limit* of the mutation rate was obtained. This estimate is 5×10^{-6} mutations per locus (loci, *if* there are several) per generation. An estimate of the lower limit could not be made.

These and other findings are discussed. The reasons for the higher estimates of relative fitness reported in other studies of H.c. are considered.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Dr. Friedrich Vogel and Dr. William J. Schull for their comments.

REFERENCES

BELL, JULIA. 1934. *The Treasury of Human Inheritance.* Vol. 4. Nervous diseases and muscular dystrophies. Part 1. Huntington's chorea. Cambridge: Cambridge University Press.

BROTHERS, C. R. D. 1949. The history and incidence of Huntington's chorea in Tasmania. *Proc. R. Australas. Coll. Physicians* 4: 48–50.

COCHRAN, W. G. 1953. *Sampling Techniques.* New York: Wiley and Sons.

CROWE, F. W., SCHULL, W. J., AND NEEL, J. V. 1956. *A Clinical, Pathological, and Genetic Study of Multiple Neurofibromatosis.* Sprinfigeld: C. C Thomas.

ENTRES, J. L. 1921. Über Huntingtonsche Chorea. *Zschr. ges. Neur. Psychiat.* 73: 541–551.

KISHIMOTO, K., NAKAMURA, M., AND SOTOKAWA, Y. On population genetics of Huntington's chorea in Japan. *Proc. First Internat. Cong. Neurol. Sc.*, Brussels, 1957. (In press.)

KROOTH, R. S. 1955. The use of the fertilities of affected individuals and their unaffected sibs in the estimation of fitness. *Am. J. Human Genet.* 7(4): 325–359.

PALM, J. D. 1953. *Detection of the Gene for Huntington's Chorea.* Ph.D. Thesis. University of Minnesota.

PANSE, F. 1942. *Die Erbchorea.* Leipzig: Thieme.

REED, S. C. AND PALM, J. D. 1951. Social fitness versus reproductive fitness. *Science* 113: 294–296.

REED, T. E. 1957. A genetic survey of Huntington's chorea in the state of Michigan. Proc. Symp. Twin Research Pop. Genetics, Tokyo, 1956. Supplement, *Jap. J. Human Genet.* 2: 48–53.

REED, T. E. 1959. The definition of relative fitness of individuals with specific genetic traits. *Am. J. Human Genet.* 11: 137–155.

REED, T. E. AND CHANDLER, J. H. 1958. Huntington's chorea in Michigan. 1. Demography and genetics. *Am. J. Human Genet.* 10: 201–225.

REED, T. E. AND NEEL, J. V. 1955. A genetic study of multiple polyposis of the colon (with an appendix deriving a method of estimating relative fitness). *Am. J. Human Genet.* 7: 236–263.

VOGEL, F. 1957. Neue Untersuchungen zur Genetik des Retinoblastoms (Glioma retinae). *Zschr. menschl. Vererb.* 34: 205–236.

WHELPTON, P. K. 1954. *Cohort Fertility.* Princeton: Princeton University Press.

WITKOP, C. J. 1957. Hereditary defects in enamel and dentin. *Acta Genet. et Stat. Med.* 7: 236–239.

The Definition of Relative Fitness of Individuals With Specific Genetic Traits[1]

T. E. REED

Department of Human Genetics, University of Michigan Medical School, Ann Arbor

INTRODUCTION

THE CONCEPT of relative biological (Darwinian) fitness or selective value, loosely defined as the fertility of one class of individuals relative to that of another, is well established in human genetics. This relative fertility has customarily been expressed as the ratio of mean numbers of children (Haldane, 1935) and this is probably the best simple comparison of natural selective values which can be made. This ratio is clearly implied in the parameter W (for relative fitness), when mathematically describing the effects of selection on the gene frequency of a particular gene, since we are expressing the relative proportion of genes in one generation which are transmitted to the following generation. Considering its importance, it is surprising that no exact, practical definition of relative fitness for use in human populations has been devised.

In a recent extensive study of Huntington's chorea (Reed and Chandler, 1958; Reed and Neel, 1959), an attempt to measure the relative fitness of individuals heterozygous for the dominant gene for Huntington's chorea revealed several important deficiencies in the usual method of estimating W. One important finding was that the normal sibs of choreics differed significantly in mean fertility from the general population so that the usual practice of comparing affected individuals with their normal sibs could not be used. This finding, therefore, challenges the validity of the common assumption that normal sibs of affected individuals are representative of the general population. This assumption does not appear to have been tested for other traits. Other realizations of possible deficiencies of sib comparisons were the consequence of the relatively high W for Huntington's chorea, around 0.8, since it was apparent that biases, such as age differences between affected and normal individuals, which were negligible when W was low, became important as W approached one. This paper is an attempt, growing out of the above-mentioned study, to define W for specific genetic traits more precisely. A new definition of relative fitness will be proposed and its calculation illustrated. These purposes seem best served by considering in turn (1) the "reference population" used in estimating W (the population whose fitness is compared with that of the trait in question) and (2) the methods of estimating W.

Received July 5, 1958.

[1] This study was supported in part by Contract AT (11-1)-405 of the United States Atomic Energy Commission.

THE REFERENCE POPULATION

The reference population may, of course, be any population in which one is interested. If one is specifically interested in comparing the fertility of trait-bearers with that of their normal sibs, then the normal sibs are the reference population. However, for the population genetics of specific genetic traits, such as traits determined by rare dominant genes, the reference population should (unless indicated to the contrary), be the general population itself. This is obvious, since all of the other parameters, such as frequency and rate of mutation from the normal allele to the mutant allele, are with reference to the general population. However, since data on the general population are almost never used, it is well to emphasize this fact. The basic problem, then, is how to obtain fertility data on the general population. The choice will usually be between (a) using government census data on women classified simultaneously by age and by number of children ever born or (b) utilizing small sample surveys, usually made for other purposes. In the past (b) has been followed and the normal sibs of the trait-bearers have been used as a sample of the general population. The implicit reason for choosing (b) is usually either that no adequate population data exist or that, even if they do, the possible biases in the sample are unimportant. This latter belief will always be justified when W approaches zero but, as the example for Huntington's chorea shows, it is not necessarily true when W is not low.

The only study using census data for the reference population appears to be this above-mentioned study of Huntington's chorea. U. S. Census fertility data for the state of Michigan, U. S. A., for 1940 were used, this being the year for which a retrospective statewide census of choreics was made. The number of liveborn children ever born per woman (married or unmarried), classified by 5-year age groups, was the available measure of fertility. A direct comparison, within age groups, is therefore possible for any Michigan sub-population living in 1940. Data on age-specific cumulative fertility for total women, such as were used here, are available in many countries. In the United States, for example, these data are available for the years 1910, 1940, and 1950, for the entire nation and for individual states. These data are usually obtained on a certain small fraction of women enumerated and consequently there is a small sampling error. In these Michigan data the standard error is about 0.05, about two per cent of the mean. Since the data are for females the question arises of their suitability for representing the entire population. As is discussed later, the *completed* fertility is what is desired so that we need consider only individuals who have reached some arbitrary age near the end of the reproductive period, say 45 years. The age-specific fertility differences between males and females then become negligible. It is clear that the mean number of children *ever* born per female cannot differ greatly from the corresponding mean for males in societies where the marriage rates and numbers of the two sexes are similar. Direct evidence for this approximate equality was found for the normal sibs of choreics by Reed and Neel (1959) and also for the normal sibs of individuals with neurofibromatosis, using the original data of Crowe, Schull, and Neel (1956). This latter calculation is presented below. These census data on completed fertility of females are therefore suitable for representing the entire population.

An important deficiency, for genetic purposes, of most census fertility data, is

that they are given for *surviving* women. As is discussed below, the most informative measure of fitness, taking into account both adult fertility and viability from birth to adulthood, is mean number of livebirths ever (to be) born per (liveborn) *newborn* individual, defined here as $\bar{B}_o$. The corresponding mean per woman surviving to (or near to) the end of reproduction is here called $\bar{B}_s$. $\bar{B}_o$ is not usually available. It is not available, for example, for any representative United States sub-population. In this one respect the use of normal sibs may offer an advantage over the usual census fertility data since it is possible, for traits recognizable at, or soon after birth, to estimate this quantity. However, depending on the nature of the trait, and the available census fertility data, estimates of $\bar{B}_s$ for two populations, *1* and *2*, may be usable to obtain valid estimates of the true relative fitness. It can be shown that this will be so (i.e., $\bar{B}_{1\cdot s}/\bar{B}_{2\cdot s} = \bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$) when (a) the proportions of newborn individuals in *1* and *2* surviving to the end of reproduction are equal and (b) $\bar{B}_{1\cdot s}/\bar{B}_{2\cdot s} = \bar{B}_{1\cdot d}/\bar{B}_{2\cdot d}$, where $\bar{B}_d$ is the mean fertility of individuals dying before the end of reproduction. When a trait decreases viability (a) is not true and this comparison is not valid. In general, comparison of normal sibs of affected persons with census fertility data should be valid, $\bar{B}_{1\cdot s}/\bar{B}_{2\cdot s}$ giving a good estimate of $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$. Census fertility data on deceased women are not available but Reed and Neel (1959), using the data on American white native-born women in *Cohort Fertility* (Whelpton, 1954), found that for women born in 1890 only about 10 per cent of the number of children ever born per newborn were born to women dying before age 47. Therefore, if (a) is true, but (b) is not, the maximum error is not over about 10 per cent; the actual error should be appreciably smaller. For workers in the United States *Cohort Fertility* enables one to approximate $\bar{B}_o$ for women born around 1900 or slightly earlier. (I am indebted to P. K. Whelpton, Scripps Foundation for Research in Population Problems, Miami University, Miami, Ohio, for valuable suggestions for estimating $\bar{B}_o$). This procedure, described in Reed and Neel (1959), requires making an assumption about the unknown cumulative fertility of women dying in the reproductive period. The assumption made was that the mean cumulative fertility of women dying at age x, say 30 years of age, is the same as that of women living at age x. This is obviously untrue in certain cases, such as women dying from chronic disease, but this effect may be offset by the greater mortality and fertility of the lower socio-economic groups. At any rate, as discussed above, the maximum error is about 10 per cent. An indication that the estimate obtained by this procedure is reasonable is found by comparing the estimate of 2.08 children ever born per white Michigan woman born in 1890 with the value (calculated in this paper) of 2.037 ± 0.240 for 107 normal Michigan sibs of sporadic cases of neurofibromatosis, reaching age 40 or over, or dying at any age, using the original data of Crowe, Schull, and Neel (1956). In other countries special fertility data may also be available for the estimation of $\bar{B}_o$.

When the reference population is the general population, the choice of method for approximating $\bar{B}_o$ will vary with the trait and the country. In deciding between census fertility data and normal sib fertility data, the fact that the census gives $\bar{B}_s$ instead of $\bar{B}_o$ must be weighed against three disadvantages of the sib comparison: 1) The true fertility of the normal sibs may differ from that of the general population, 2) the estimate of sib fertility will usually have a much larger standard error than that

of the census, and 3) the method of ascertainment may bias the fertility estimate of the normal sibs. Possibility 1) has already been illustrated and 2) is obvious. Perhaps the most obvious example for 3) is bias for large sibships, which, because of the small positive correlation between sibship size and fertility of normal individuals (Pearson, Lee, and Bramley-Moore, 1899; Fisher, 1935), would increase the observed fertility of the normal sibs. This correlation has not been clearly established for normal individuals in populations studied after 1900, but, as shown below, it probably exists. Krooth (1955) has devised a method to correct (partially) for this bias but, as will be shown, his method is open to other objections.

METHODS

In this section a new definition of relative fitness will be proposed and illustrated. Other definitions which have been advanced will also be considered.

What to measure

Because of the unlimited number of aspects of fertility which may be considered, and the considerable number which have already been used, agreement on *what* to measure seems necessary before the methods of using these measurements can be discussed. It is suggested here, as a self-evident fact, that livebirths and not total births (livebirths plus stillbirths), are the preferred units of fertility. A stillbirth makes no contribution to the gene pool of its generation. Census data, it may be noted, are usually in terms of livebirths.

1. Time span of fitness measurement: It is suggested that (with the exceptions discussed below) the most meaningful measure is mean number of livebirths ever (to be) born per *newborn* (liveborn) individual. This has previously been defined as $\bar{B}_o$. By expressing the measure in terms of the ultimate fertility that a newborn will, on the average, have by the end of his reproductive period, we get an overall measure of all factors affecting his ability to contribute genes to the next generation. By using number of children ever born, which in practice means studying individuals who have either (a) reached some arbitrary high age, say 40 or 50 years, or (b) have died at any age (we ask concerning each individual whether he is includable under (a) or under-(b); we do *not* ask with regard to (a) only or to (b) only since this would introduce a bias), we have complete information on his genetic performance, which is not the case if he is still in his reproductive period. This use will also eliminate bias due to age differences between the samples being compared. It is obvious that it is entirely incorrect to determine the mean fertility of a group of individuals of widely differing ages, without specifying age, and then compare this mean with that of another group, also unspecified for age. A child of age 10, for example, with zero children, would be regarded as equal in fertility to a married individual of age 50 years having no children, even though this child may have eight children when he attains the age of 50. It should be noted that for certain conditions having a late onset, there will be a bias for higher ages in propositi than in their normal sibs. This higher mean age will spuriously raise the observed fertility of affected individuals relative to the normal when incompleted fertilities are used. The use of completed fertility in estimating relative fitness was recommended by Haldane (1935) and has been practiced by a

number of workers but ignored by others. Krooth (1955), for example, does not consider it necessary to note the age of individuals whose fertility is being measured.

2. Time unit of the rate of fertility: It is not always obvious that the number of children born is in fact a *rate* of birth. If $\bar{B}_o$ is used, this is actually the number per generation, a generation being about 29 years (mean of all individuals, ignoring sex) in normal Western populations. It is obvious that if the length of generation differs between the two groups being compared, number per generation is an inexact, possibly misleading, measure of fitness. A simple way to avoid this difficulty is to express fertility in mean livebirths per newborn individual *per year*, instead of per generation. This procedure is illustrated below. It will be seen that the actual parental age distribution, not the mean generation length (= mean parental age) is involved.

3. Type of information to use in estimating relative fitness: The data one ideally wishes to have, for each group being studied, are, clearly, the complete life history of a large random sample of newborn liveborn individuals. $\bar{B}_o$ can then be calculated and, if age at the birth of each livebirth is also noted, livebirths per year can also be calculated. This type of data is approached when an exhaustive survey of all known cases of a trait is made for a given area at a given time, and information is also obtained on all sibs of these propositi. Because of secular changes in fertility, the data used should be kept homogeneous in time as much as is feasible without sacrificng too much data. Then, from all affected individuals, including propositi, in the sibships of the propositi, those liveborn persons who have completed their reproduction, either (a) by reaching some arbitrary high age or (b) by dying at any age, can be picked out. The total number of livebirths born to these individuals divided by their number is then $\bar{B}_o$. All individuals satisfying (a) or (b) are used, including those dying very young. It is clear that if one required only (a) there would be a bias for including the more viable, and probably more fertile, of the affected persons, while if only (b) were required there would be a bias for the more inviable. The same procedure can be used for the normal sibs. The use of the normal sibs as a reference population must be considered in the light of the previous discussion.

Unfortunately, data are often not from complete surveys and in this situation the possible effect of method of ascertainment on the estimate of fitness must be considered. A comprehensive examination of biases due to ascertainment would include those present in complete as well as in single (non-exhaustive) ascertainment. However, since at least 30 possible situations (combinations of: single or complete ascertainment, sporadic [both parents normal] or familial or both kinds of affected sibs, ditto for normal sibs, comparisons of affected vs. normal sibs, or normal sibs vs. general population, or affected sibs vs. general population) exist in which bias might arise, a comprehensive treatment will not be attempted. Instead, we may note that there are three principal possible biases which may operate in some of these 30 fertility comparisons, (a) the probability of ascertaining a sibship (in single ascertainment) is proportional to sibship size, s, (b) the probability that, for a dominant trait, among sibships having an affected parent, the probability of ascertaining a sibship of size s is proportional to $[1 - (1/2)^s]$, and (c) the fertility of normal sibs is altered because of having an affected parent or affected sib. Bias due to (a) or (b) requires a correlation between s and fertility. Bias (a) has already been mentioned

and the consequences of (b) have been given by Slatis (1955), namely, a bias for larger s in normal sibs than in the affected individuals. Bias (c) is illustrated by the findings of Reed and Neel (1959) in Huntington's chorea. Bias (a) occurs in single (incomplete) ascertainment but not in complete ascertainment, while (b) occurs in both single and complete ascertainment. If bias (c) exists, it will, of course, be independent of ascertainment.

Since no estimates of the magnitude of biases (a) or (b) have been made, it would seem worthwhile to estimate them. Because (a) will represent an increased bias in single ascertainment, relative to complete ascertainment, and because the calculation is simpler and more easily interpreted, this bias is estimated in the appendix. Although tedious, (b) could, in theory, also be calculated in a similar way, but in fact we need to know the frequency distribution of sibship sizes from matings of affected by normal as well as whether bias (c) is present. The calculation shows that for a mean population fertility of two children ever born, a linear regression of fertility on sibship size of +0.1 (a reasonable value), the bias in observed mean fertility due to (a) is +0.176. Bias due to (b) however, should be appreciably less than this since $[1 - (1/2)^s]$ asymptotically approaches one with increasing s, while bias in (a) depends directly on s, having no upper limit as s increases. With the variances in number of livebirths ever born usually observed, around six, 0.176 will be less than the standard error for samples of less than about 200. Without appreciable error, this bias may be neglected for these sample sizes. This neglect seems more justifiable when it is considered that, in practice, the precise manner of ascertainment is rarely known or is a mixture of several types, often single and multiple ascertainment.

Krooth (1955) devised a method to correct for bias (a) in comparing fertility of normal and dominantly affected sibs ascertained by single ascertainment. Bias (b), however, is not corrected when, as he suggests, the fertility of all affected sibs (sporadic and familial) is compared with the fertility of sporadic unaffected sibs. This bias is corrected for in his method for recessive traits. The absence of sporadic normal sibs in sibships of size one is an additional bias, as Krooth points out.

In summary, it seems fair to conclude that while biases deriving from the small correlation between sibship size and fertility exist in all calculations of relative fitness, they are less than the standard error of the mean fertility for sample sizes under about 200 and may be neglected. In more extensive data, it would probably be worthwhile to calculate the known biases and then correct the fertility estimates accordingly.

A definition of relative fitness based on mean number of livebirths ever born per newborn per year (B_y)

To eliminate the bias due to differences in the parental age distributions, discussed above, the following definition of relative fitness (W) is proposed: Let

x = age in years at last birthday. (On the average, this will be 0.5 years less than the exact chronological age but the small resulting bias appears in both numerator and denominator and should very nearly cancel out.)

N_i = original number of liveborn newborn individuals of genotype i whose completed reproduction is known.

$B_{i\cdot x}$ = number of livebirths born to the survivors of the N_i individuals during their xth year of life.

$\bar{B}_{i\cdot o}$ = mean number of livebirths ever born per newborn liveborn individual of genotype i.

$B_{i\cdot y}$ = mean number of livebirths ever born per newborn liveborn individual of genotype i per year, based on $\bar{B}_{i\cdot o}$ livebirths ever born.

$P_{i\cdot x}$ = parental age frequency distribution of the N_i individuals (the proportion of livebirths, out of $\bar{B}_{i\cdot o}$ livebirths, which is born to the survivors of these individuals at age x).

Then, from these definitions,

$$\bar{B}_{i\cdot o} = \frac{1}{N_i}\sum_x B_{i\cdot x}\,,\quad P_{i\cdot x} = \frac{B_{i\cdot x}}{\sum_x B_{i\cdot x}}\,,\quad B_{i\cdot y} = \frac{1}{N_i}\sum_x \frac{B_{i\cdot x}}{x}\,.$$

If we are estimating the fitness of population *1* relative to that of population *2*, the following definition will compare the true annual birth rates:

$$W = \frac{B_{1\cdot y}}{B_{2\cdot y}}. \tag{1}$$

From the above definitions,

$$W = \frac{N_2\sum_x \dfrac{B_{1\cdot x}}{x}}{N_1\sum_x \dfrac{B_{2\cdot x}}{x}} = \frac{\bar{B}_{1\cdot 0}\sum_x \dfrac{P_{1\cdot x}}{x}}{\bar{B}_{2\cdot 0}\sum_x \dfrac{P_{2\cdot x}}{x}}. \tag{2}$$

Since the customary definition of W, ignoring parental age distribution, is $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$, the effect of the parental age distribution is clearly seen. It should be noted that it is the actual distribution, not the mean parental age, which is important. A simple numerical example of these definitions may be given. If individuals of genotypes *1* and *2* have no deaths between births and the end of reproduction and, on the average, have three livebirths ever born, *1* having them at exact ages 20, 25, and 30 years, and *2* at 30, 35, and 40 years, then

$$B_{1\cdot y} = \sum_x \frac{B_{1\cdot x}}{x} = \frac{1}{20} + \frac{1}{25} + \frac{1}{30} = 0.123$$

$$B_{2\cdot y} = \sum_x \frac{B_{2\cdot x}}{x} = \frac{1}{30} + \frac{1}{35} + \frac{1}{40} = 0.087$$

and, from equation (1)

$$W = \frac{0.123}{0.087} = 1.41$$

instead of unity if parental age distribution is ignored. It is obvious that *1* will increase faster than *2* and, in time, would supplant *2*, even though $\bar{B}_{1\cdot o}$ equals $\bar{B}_{2\cdot o}$.

For this reason equation (1), or its equivalent (2), is proposed as the general definition of relative fitness because it compares the mean genetic contribution per year.

It is evident that if $\sum_x \frac{P_{1\cdot x}}{x} = \sum_x \frac{P_{2\cdot x}}{x}$, or more particularly, if $P_{1\cdot x} = P_{2\cdot x}$ for all x, equations (1) and (2) reduce to the usual ratio of means. If it is known, or probable, that the parental age distributions of *1* and *2* do not differ, $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$ is then correct. It should be noted, however, that for certain human genetic traits, especially those having onset in the reproductive period, there is an *a priori* expectation that the parental age distribution will be altered. Thus, in multiple polyposis of the colon the mean age at appearance of clinical symptoms is around 30 years and it is certain that some individuals affected with this disease have their reproduction terminated early. Their livebirths, then, have a lower mean parental age, and, consequently, greater genetic value, than those of the general population. For such diseases it may be necessary to use equation (1) even though a clear difference between parental age distributions cannot be demonstrated.

An example of estimating W *using* $\bar{B}_o$ *and* B_y

The data of Crowe, Schull, and Neel (1956), together with their unpublished data, on a survey in the state of Michigan of multiple neurofibromatosis, a rare dominantly inherited disease, are suitable for illustrating the estimation of W both by (1) and by $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$. The estimates obtained are of interest in themselves for two reasons. This disease appears to have the highest mutation rate for any human dominant trait (Crowe *et al.*, 1956), about 10^{-4} mutations per haploid genome per generation, as estimated both by direct and indirect (using relative fitness) methods. Crowe *et al.* used the method proposed by Krooth (1955) for calculating W and therefore it is of interest to compare their estimate for W of 0.527 with that obtained by the above methods.

The study of Crowe *et al.* was carried out in the years 1950–1953 and is, in large part, based on the sibships of patients diagnosed at University Hospital, Ann Arbor, Michigan between 1934 and 1953. The ascertainment of affected individuals is not complete for any specified area and, with seven exceptions, kindreds (groups of related individuals) were ascertained only once (single ascertainment). The probability of inclusion of a kindred in this study is therefore approximately proportional to the number of affected members living in the above time interval (as opposed to number of affected individuals in a sibship, since a kindred may be ascertained through more than one sibship). A similar probability applies to the ascertainment of sibships (Weinberg, 1913; Greenwood and Yule, 1914). As Krooth (1955) has pointed out, the probability for single, non-exhaustive ascertainment, of ascertaining either a "sporadic" sibship (parents normal) or a "familial" sibship (one parent affected) is proportional to sibship size (if ascertainment is only through the sibship and not through relatives). As is shown in the appendix, when ascertainment is of this type and the regression of individual fertility on sibship size is +0.1 (mean for males and females), the bias in the estimate of mean fertility of normal individuals is less than the standard error for sample sizes smaller than about 200. Since 59.5 (the fractional number

TABLE 1. COEFFICIENTS OF REGRESSION OF INDIVIDUAL FERTILITY (NUMBER OF LIVEBIRTHS) ON SIBSHIP SIZE OF THE INDIVIDUAL. DATA OF CROWE ET AL. (1956). ONLY INDIVIDUALS LIVING TO AGE 40 YEARS OR MORE OR DECEASED AT ANY AGE. MARITAL STATUS IGNORED

"Affected" = has multiple neurofibromatosis
"Unaffected" = normal sib of an affected person

Type of propositus of the kindred	Individual	Sex	Number	Regression	Standard error
Sporadic	Affected	Both	23	−0.123	0.141
	Unaffected	Males	46	+0.089	0.115
		Females	42	+0.398**	0.159
		Both	88	+0.222*	0.096
Familial	Affected	Both	23	+0.078	0.070
	Unaffected	Both	23	−0.174	0.205

** Significant at the 0.02 level.
* Significant at the 0.05 level.

is explained below) affected individuals and 107 normal sibs are available for estimating $\bar{B}_o$, this bias may be neglected.

Although the numbers available for the data of Crowe *et al.* are small, it is of interest to calculate these fertility regressions directly from their data because there appear to be no published data on the regression of individuals affected with a disease, or on fertility as measured by number of livebirths ever born, counting from the birth of an individual. These regressions were calculated for the same individuals used in estimating W and are given in Table 1. It was thought advisable to separate the "sporadic" normal sibs from the "familial" normal sibs because the former should be more representative of the general population. It is seen that only (a) sporadic normal females and (b) sporadic normal individuals (males plus females) have significant regressions, being $+0.398 \pm 0.159$ and $+0.222 \pm 0.096$ respectively. The latter does not differ significantly from the value of $+0.1$ used in the appendix, chosen for being nearer the results obtained by Pearson, Lee, and Bramley-Moore (1899) and Fisher (1935) from much more extensive data. Of particular note is the finding that there is no suggestion of a significant regression in affected individuals. It therefore appears that a significant regression occurs in the general population of Michigan but cannot be demonstrated among the affected individuals of this study. The numbers are too small to draw firm conclusions, but these findings seem reasonable if, as is the case for neurofibromatosis, the disease seriously interferes with fertility. (This follows from the fact that variance in number of livebirths born to affected individuals is determined by the varying effect of the disease on fertility, in addition to the effects of sibship size and "all other factors," while in the normal sibs only the latter two quantities are operating. Therefore the proportion of the total variance due to sibship size and consequently, the correlation, is smaller in affected individuals than in normals.)

In deciding which individuals to use for estimating fitness, the guiding principle was to obtain from the data as large a number as possible of affected individuals and their unaffected sibs whose completed reproductive performance was known and whose ascertainment was independent of viability and fertility. (The use of unaffected sibs

for a reference population is, of course, dependent on the demonstration that they are satisfactorily representative of the general population with regard to fitness.) It is not claimed that this latter goal is completely fulfilled but it is believed that major biases are absent. Some selection for severity of the disease probably occurred but, countering this, is the fact that about 27 per cent of the propositi were ascertained because they were seen for some entirely unrelated complaint and an additional 18 per cent were seen only because of cosmetic effects of neurofibromatosis. Also, all affected sibs of propositi meeting the above qualifications, are included, and there should be little bias among these. Sibships containing affected individuals selected only through an affected child were excluded, as were sibships with non-Michigan propositi and those with incomplete diagnostic or reproductive histories. Only individuals who, at the time of the study, were 40 or more years of age, or who had died at any age, were included. Therefore reproduction was nearly, or completely, ended. A higher minimum age for the living would be desirable but would reduce the numbers of usable individuals. Because neurofibromatosis may not be recognizable until several years after birth, a convention is necessary regarding the scoring of the apparently normal sibs in "familial" kindreds who died in infancy. Seven males and two females died under one year or "in infancy" in these kindreds. Since one parent was affected, half were counted as affected and half as normal. In the "sporadic" kindreds, since the parents and other ancestors were normal, these early deaths were counted as normal. In Table 2 the distribution of affected (sporadic plus familial) individuals and the normal sibs of sporadic affected individuals, by number of livebirths ever born to them, is given. The striking sex differences in fitness of affected individuals, noted by Crowe *et al.*, is apparent, males being less fertile than females. Since the number of affected males and affected females in the sample differ, while in the general population the numbers of males and females at birth is approximately equal, it is necessary to estimate the mean affected fertility as the unweighted mean of the values of the two sexes. This mean is 0.872 ± 0.191 livebirths ever born. The mean for normal sibs of sporadic propositi (the sexes do not differ so all individuals may be pooled) is 2.037 ± 0.240. There is therefore clear evidence that the fitness of affected individuals is less than that of these normal sibs. The conventional estimate of fitness, $\hat{B}_{1\cdot o}/\hat{B}_{2\cdot o}$ would be $0.872/2.037 = 0.43 \pm 0.11$. For the *living* normal sibs Table 2 also shows that $\bar{B}_x$ does not differ significantly from the mean expected if the age-specific cumulative fertility is the same as that of white females in Michigan in 1950 (U. S. Census data). This is evidence that these normal sibs of sporadic propositi are representative in their fertility of the general population and are a suitable reference population. The total number of normal sibs of affected in the "familial" kindreds, 39.5, is too small to warrant a separate distribution but it may be noted that the mean for all such sibs is 1.468 ± 0.337. This is appreciably, but not significantly, smaller than the mean for all normal sibs of sporadic affected. These two types of normal sibs are not pooled because there may be a real difference between them. It seems probable that the normal sibs of sporadic propositi, because they have normal parents, may resemble the general population in fertility more closely than the "familial" normal sibs.

TABLE 2. DISTRIBUTION OF INDIVIDUALS BY NUMBER OF LIVEBIRTHS EVER BORN. INDIVIDUALS LIVING TO AGE 40 YEARS OR MORE OR DECEASED AT ANY AGE. MARITAL STATUS IGNORED

"Living" = living at time of study, 1950–1953

Number of Livebirths Ever Born	All Affected		Normal Sibs of Sporadic Propositi					
			Males			Females		
	Males	Females	Living	Deceased	Total	Living	Deceased	Total
0	29.5*	13	9	10	19	11	10	21
1	2	3	4	0	4	6	1	7
2	2	2	15	1	16	9	0	9
3	0	4	7	2	9	3	1	4
4	0	0	2	0	2	2	0	2
5	2	1	2	0	2	2	0	2
6	0	0	1	0	1	1	0	1
7	0	1	1	0	1	1	0	1
8	0	0	0	0	0	2	0	2
9	0	0	1	0	1	1	0	1
10	0	0	1	0	1	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0	0	0	1	0	1
N = No. of individuals	35.5*	24	43	13	56	39	12	51
No. of livebirths	16	31	105	8	113	101	4	105
$\bar{B}_o$ = Mean no. of livebirths	0.451	1.292	2.442	0.615	2.018	2.590	0.333	2.059
S.E. of mean	0.208	0.383	0.348		0.295	0.470		0.387
Expected mean no. of livebirths**	—	—	2.358	—	—	2.377	—	—

Mean no. of livebirths for individuals, ignoring sex:

All affected (mean of value for males and value for females): 0.872 ± 0.191.

All normal sibs of sporadic propositi (all individuals pooled): 2.037 ± 0.240.

* Unaffected individuals in "familial" sibships dying in "infancy" or under one year are counted as one-half affected and one-half normal.

** Expected if age-specific cumulative fertility is same as that of white women in Michigan in 1950. Data of 1950 U. S. Census.

The parental age distribution is needed for the calculation of $\sum_x \frac{B_{i \cdot x}}{x}$ and this is given in Table 3 for all the individuals of Table 2 whose parental ages are all known. Since the importance of parental age was not stressed at the time these data were collected, it was not always available, especially for normal sibs, and a correction becomes necessary. Only liveborn children from individuals all of whose ages at the births of these children were known, were used. If the number of these livebirths for a specified group of individuals is B_k and the number of livebirths from individuals all of whose parental ages are *not* known is B_u, then

$$\sum_x \frac{B_{i \cdot x}}{x} = \left(1 + \frac{B_u}{B_k}\right) \sum_x \frac{B_{k \cdot x}}{x},$$

TABLE 3. DISTRIBUTION OF PARENTAL AGES. LIVEBIRTHS TO INDIVIDUALS IN TABLE 2 WHOSE AGES AT BIRTH OF A LIVEBORN CHILD ARE ALL KNOWN. SEE TEXT FOR SYMBOLS

Age x	All Affected		Normal Sibs of Sporadic Propositi		Age x	All Affected		Normal Sibs of Sporadic Propositi	
	Males	Females	Males	Females		Males	Females	Males	Females
17	0	0	0	2	40	0	1	0	1
18	0	0	0	1	41	1	0	1	1
19	0	0	0	1	42	0	1	2	0
					43	0	0	1	0
20	0	1	1	1	44	0	1	1	0
21	1	2	0	2					
22	2	0	1	5	45	0	0	0	0
23	1	3	1	3	46	0	0	1	0
24	2	1	2	2	47	0	0	2	0
25	0	1	2	9					
26	0	1	3	0					
27	1	1	1	4	$\bar{x}$	28.43	30.31	32.92	28.08
28	2	1	4	6					
29	0	0	3	5	S.E. of $\bar{x}$	1.73	1.35	0.97	0.70
30	0	3	4	4	B_k	14	26	50	66
31	0	1	2	5					
32	1	1	1	2	B_u	2	5	63	39
33	1	2	2	2					
34	1	1	3	2					
35	0	1	1	3	$\Sigma \frac{B_{k \cdot x}}{x}$	0.514	0.902	1.585	2.452
36	0	0	4	1					
37	0	1	2	1					
38	0	2	3	2	$\Sigma \frac{B_{i \cdot x}}{x}$	0.587	1.075	3.582	3.901
39	1	0	2	1	(Estimated)				
					B_y	0.0165 (0.0307 for mean of sexes)	0.0448	0.0640 (0.0703 for mean of sexes)	0.0765

where $B_{k \cdot x}$ refers to the B_k livebirths. This is correct if the parental age distribution for B_k and B_u are the same or very nearly so. This should be true in most cases. The mean parental ages of affected individuals, 28.43 ± 1.73 years for males and 30.31 ± 1.35 years for females, do not differ significantly from the corresponding means of the normal sibs. The mean parental age for *sporadic* affected females (not given separately in the table), however, was 35.00 ± 1.48 years and does differ significantly from the mean for normal females, 28.08 ± 0.70. The values for the general population of Michigan in 1935 (Data of the U. S. Bureau of the Census: Vital Statistics—Special Reports), when many of the individuals in Table 3 were reproducing, are 31.559

years for males and 27.203 years for females. The observed mean for affected males does not differ significantly from the mean for Michigan fathers, but that for affected females does differ from the mean for Michigan mothers at the .05 level. The maternal age distribution of affected females therefore appears to differ from that of normal females and, in consequence, $B_{i\cdot y}$, not $\bar{B}_{i\cdot o}$, should be used. (If the numbers of affected individuals were larger, a better test would be a comparison of observed and expected numbers for convenient age groups.)

The values obtained from Table 3 for the mean number of livebirths ever born per newborn (liveborn) individual per year (B_y) are:

	Males	Females	Mean of males and females
All affected	0.0165	0.0448	0.0307
Normal sibs of sporadic propositi	0.0640	0.0765	0.0703

Then, by eq. (1)

$$W = \frac{B_{1\cdot y}}{B_{2\cdot y}} = \frac{0.0307}{0.0703} = 0.44,$$

denoting affected individuals as population *1* and normal sibs as *2* (the reference population). This is near the value of 0.43 obtained from $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$ but lower than the estimate of 0.53 obtained by the method of Krooth (Crowe *et al.*, 1956). Possible reasons for the difference (which is not statistically significant) between the estimate of 0.44 and that of Krooth are discussed below. Because $\bar{B}_{1\cdot o}$ is very significantly different from $\bar{B}_{2\cdot o}$ we are quite sure that $B_{1\cdot y}$ is significantly different from $B_{2\cdot y}$ and, therefore, that the resulting W is significantly different from unity. If this were not the case the standard errors of $B_{1\cdot y}$ and $B_{2\cdot y}$ could be calculated from the $\sum_x \frac{B_x}{x}$ of each of the N_i individuals, although this would be somewhat tedious. In general, significance tests should be applied to estimates of W as for any other estimated parameter. This practice, however, usually has not been followed.

The important question of what to do when the unaffected sibs can be shown to differ in fertility from the general population, as is the case for Huntington's chorea (Reed and Neel, 1959), must be considered. The first point to note is that, for traits affecting viability, it is incorrect to compare the fertility of *living* affected individuals with that of the living members of the general population because this would introduce a bias for mildly affected persons. One can either obtain an estimate of $\bar{B}_o$ for the general population from other sources or one can adopt a procedure used by Reed and Neel (1959). If $W_{a\cdot n}$ is the fitness of affected individuals relative to that of their normal sibs (in terms of $\bar{B}_o$ or B_y) and if $W_{n\cdot p}$ is the fitness of the normal sibs relative to that of the general population (in terms of $\bar{B}_z$, mean number of livebirths of living individuals, as of a given date, who have completed their reproduction), then, to a good approximation,

$$W = (W_{a\cdot n})(W_{n\cdot p}).$$

Since the mutation rate, μ, of a rare dominant trait is $f(1 - W)/2$, where f is the frequency of the trait at birth and the population is in genetic equilibrium, the indirect estimate of μ for neurofibromatosis is increased by a factor of $(1 - .44)/$

$(1 - .53) = 1.2$ when the value of 0.44 is used instead of the value found by Crowe *et al.* The value of f is only approximately known, being $3\text{–}4 \times 10^{-4}$, so that this change is unimportant. When W approaches unity, however, small differences in the estimates of W are seen to have large effects on the estimate of μ.

Other methods of estimating W

A number of other methods have been proposed, and used, for estimating relative fitness but they use less of the potentially available information about fitness and therefore, explicitly or implicitly, make more assumptions. They may be useful and even essential, depending on the data, but their use is justified only because complete reproductive information, from birth to end of reproduction, is not available. None take into consideration the parental age distribution.

1. Method of Krooth (1955): This has already been referred to at some length. The reader should see his paper for details of the method. In general, individuals are classified by the size of their own sibship and their fertilities are combined in such a way that the comparison of affected persons with their normal sibs is, in theory, unaffected by the bias from ascertainment and correlation between sibship size and fertility. However, as previously discussed, this bias should be very small for complete ascertainment, and, for samples of less than about 200, less than the standard error when ascertainment is proportional to sibship size. Krooth's method omits sibships of size one so that its efficiency in reducing bias due to ascertainment in large samples is in doubt. For large sample size a case can be made for using $B_{1\cdot y}/B_{2\cdot y}$ or $\bar{B}_{1\cdot o}/\bar{B}_{2\cdot o}$ and then subtracting the expected bias, using the method given in the appendix for estimating this bias. Krooth's method, it should be noted, is designed for sib comparison. The problems of using normal sibs for a reference population have already been considered. Also, his method does not specify the ages of the individuals being studied. The pooling of individuals too young to reproduce with persons who have partly or completely terminated their reproduction, when estimating fertility, cannot be defended. The value of the method of Krooth would appear to depend on how these objections are met.

2. Methods measuring the fitness of only part of the life cycle: These may be conveniently divided into (a) those measuring only viability from birth to adulthood (or even a smaller time span) and (b) those measuring only the fertility of adults, ignoring possible differential viability. Since the true measure of fitness requires data on both (a) and (b), the use of either alone requires the assumption that there is no selection in the other part of the life cycle. Under (a) we may list the "Relative Reproductive Span" (*RRS*, relative survival weighted by the parental age distribution of the general population), (Reed and Neel, 1955) and the ratio of frequency of affected adults to that of affected children. *RRS* was derived for the case where there is *a priori* knowledge or likelihood that the only selection operating is a simple consequence of earlier-than-normal termination of reproduction of some affected persons at an approximately known age. It is appropriate for diseases having onset during the reproductive period. It can be used, without this prior knowledge, to estimate the selective disadvantage due to this shortening of reproduction alone, the possibility of additional selection remaining to be determined. The estimation of adult fertility

only, (b), has been used by many workers. Like (a), it may be a necessary first step toward the determination of the fitness over the whole life span.

DISCUSSION

It has been stated in this paper that the definition of choice for W is that given by equation (1), where the reference population is either the general population or some representative sample of it. Other definitions are approximations to this, being determined from fewer data. It is necessary to add, however, that the possible data giving information on fitness, and the traits to be studied, are so varied that this more exact approach may be impossible or impractical in certain instances. A less comprehensive method must then be used. General rules of procedure for all contingencies are not possible but certain desiderata which any fitness study should consider can be listed:

1. The reference population is representative of the general population (defined in time and space).
2. The data are from as large a part of the life cycle (from livebirth to end of reproductive period) as possible.
3. The possible bias due to ascertainment is either small or is corrected.
4. The parental age distributions are compared and B_y used if appreciably different.
5. The data are as homogeneous in time and in age of individuals as is feasible. In particular, only completed (or nearly so) fertilities are used.

The emphasis throughout has been on the fitness of individuals, ideally as recognizable genotypes, and not on matings. Penrose (1949) has discussed the fitness of mating types and for a number of purposes this joint fitness is of value. But it seems clear that for analysis of the transmission of genes from one generation to another it is sufficient, and simpler, to follow individuals, irrespective of their matings, and note their reproductive performance. (Complications such as non-random segregation of chromosomes or mother-child incompatibility, which could alter the expected proportions of genotypes among the children of the individuals being investigated, are ignored here. If such a mechanism is known to exist allowance must be made for its effect since it will alter the proportion of genes transmitted between generations.)

The data of Crowe *et al.* (1956) on neurofibromatosis, analyzed in the present paper, provided an example (affected females) of a group whose parental age distribution differs significantly from the general population (of females), there being an excess of births to older mothers. It is interesting to note that Reed and Neel (1959) found that females (but not males) affected with Huntington's chorea similarly differed in parental age distribution. It is not immediately apparent why there should be an excess of older mothers since the progressive nature of both diseases might be expected to reduce reproduction at higher ages. In each case this finding shows that the parental age distribution should be taken into account and that either equation (1) or equation (2) should be used.

The analysis of fertility by separate sexes should be done to test whether, as is the case in neurofibromatosis, the values of $\tilde{B}_{i\cdot o}$ differ significantly. If the sexes do not differ in fertility they may be pooled and classification by sex ignored. When there are differences and the numbers of males and females in the sample differ, the (unweighted) mean of the separate estimates of $\tilde{B}_{i\cdot o}$ or B_y should be used, since, when

dealing with traits determined by autosomal genes, this will give an estimate of the fitness of all individuals, unbiased by the sex proportions in the sample. It is noteworthy that the difference between males and females found by Crowe *et al.* (1956) for neurofibromatosis was also noted in Huntington's chorea (Reed and Neel, 1959). A discussion of this phenomenon, which may be general for genetic traits with delayed onset, is given by these authors. Penrose (1950) has shown that among the mentally defective, females are more fertile than males.

Although the parental age distribution of affected females differed from that of the general population, W estimated from equation (1) (0.44) was very near that based on $\bar{B}_{i\cdot o}$ (0.43). This is probably a consequence of the slightly higher mean parental ages of the normal sibs, relative to the general population, decreasing their value of $\bar{B}_{i\cdot y}$ and, consequently, raising that of the affected individuals. It is likely that much larger differences between these two estimates of W, statistically significant, could be obtained in some data. In such cases the importance of using equation (1) would be more obvious.

In the long run the relative fitness of a particular group should be related to the relative rate of growth it would experience if it grew only through its own reproduction (i.e., without mutation or migration). This relationship, however, is not simple. It is interesting that Fisher's (1930) Malthusian parameter, m, the relative rate of increase (or decrease) of a population, can be estimated from B_y (mean number of livebirths ever born per newborn per year) and $\bar{B}_o$ (mean number of livebirths ever born per newborn). Counting the actual contribution per parent as *one-half* the number of livebirths, the annual contribution in births that a newborn individual will make is $B_y/2$. We may also consider the deaths *per year*, D_y, that a newborn individual I will contribute to the population. D_y is equal to $B_y/\bar{B}_o$ because I is $1/\bar{B}_o$ of a (specified) parent's progeny, which are being born at the rate B_y; therefore, I can be said to be born at the rate $B_y/\bar{B}_o$. If there are no secular changes in B_y and D_y, the individual's death rate equals his (own) birthrate. Therefore

$$m = \frac{B_y}{2} - D_y = \frac{(\bar{B}_o - 2)B_y}{2\bar{B}_o}$$

This definition of m (relative increase per year) has the expected property that m exceeds, equals, or is less than zero according as $\bar{B}_o$ exceeds, equals, or is less than two livebirths ever born per newborn. Since values of m can be calculated for the two groups being studied (assuming adequate data) the relative rates of growth can be compared. However, a definition of relative fitness is not easily obtained from m_1 and m_2 (for populations *1* and *2*) because m does not approach zero when $\bar{B}_o$ does. Also, a definition of W based on m lacks the direct significance of one based on reproductive performance.

In conclusion, the critical determination of relative fitness in man will probably never be easy. As the example presented illustrates, various compromises and approximations are required. There are a number of fairly simple procedures, however, which can improve the accuracy of the estimate and it has been the goal of this paper to describe some of them.

SUMMARY

An exact, directly applicable, definition of relative genetic fitness (W) is needed in the population genetics of specific human genetic traits, but this has not yet been formulated. Commonly used estimates of W, such as the ratio of mean fertilities of affected individuals and their normal sibs, may have two important sources of error: 1) the normal sibs may not be representative of the general population in their fertility, and 2) factors which affect fitness may be omitted. An example of 1) is known. A factor affecting W which is almost always omitted from consideration is the parental age distribution (age of individuals at the birth of their children).

A new definition of W, which takes into account parental age distribution is proposed and its calculation illustrated with data of Crowe *et al.* (1956) on multiple neurofibromatosis. It is shown that when the parental age distributions in the two populations being compared are the same, the proposed definition reduces to the usual ratio of mean numbers of children ever born. Other aspects of estimating relative fitness, including use of government census fertility data, are considered.

APPENDIX

MAGNITUDE OF THE BIAS IN OBSERVED FERTILITY OF NORMAL INDIVIDUALS ASCERTAINED IN PROPORTION TO THEIR SIBSHIP SIZE

The published studies on regression of individual fertility on sibship size of the individual are based on English landed gentry and nobility (Pearson *et al.*, 1899) and on German nobility and Danzig officials (Fisher, 1935). In each study only married persons are considered. The former study found regressions of +0.05 to +0.12 for males and +0.04 to +0.22 for females, values differing according to the source and treatment of the data. The latter, based on more varied data, yielded regressions for the total of 6727 couples of +0.030 for males and +0.114 for females. All these regressions are significant.

It is possible to estimate the bias in the estimate of mean fertility due to single ascertainment for any given value of the regression coefficient, b, of livebirths on sibship size of individuals. In single ascertainment the probability of ascertaining an individual is proportional to his sibship size. This is the situation usually prevailing when, for a given area, the proportion of individuals ascertained, out of the total available, is small. If the proportion becomes high, repeated ascertainment of the same individuals begins to occur and the bias for large sibships and, consequently this bias in observed fertility, decreases, reaching zero for complete ascertainment. It is emphasized that this calculation is for *normal* individuals in all sizes of sibships. It therefore slightly underestimates the bias in comparing normal sibs with the general population. At the same time, it probably overestimates the bias in affected persons, since, as the present data indicate, b for affected persons is probably less than for normal persons. Since the mean b for males and females in the extensive study of Fisher (1935) is about +0.07, it seems appropriate to use a value of +0.1, which does not differ significantly from the higher value calculated in the present study for the data of Crowe *et al.* If, in the general population,

θ_s = frequency of sibships of size s
B_s = mean number of livebirths ever born to individuals in sibships of size s
$\bar{B}$ = mean number of livebirths ever born

and θ_s' and $\bar{B}'$ are corresponding quantities for the sample obtained by single ascertainment as mentioned above, then

$$B_s = \bar{B} + b(s - \bar{s}_i)$$

$$\bar{B} = \frac{\sum s\theta_s B_s}{\sum s\theta_s}$$

$$\bar{B}' = \frac{\sum s\theta_s' B_s}{\sum s\theta_s'} = \frac{\sum s^2\theta_s B_s}{\sum s^2\theta_s}, \quad \text{since} \quad \theta_s' = \frac{s\theta_s}{\sum s\theta_s}.$$

The first equation is the regression of fertility of an individual on his sibship size, $\bar{s}_i$ being the mean size of sibships of *individuals* in the population, equal to $\sum s^2\theta_s / \sum s\theta_s$ (to be distinguished from $\bar{s} = \sum s\theta_s$). Reasonable values for the distribution of θ_s were obtained from the 1940 U. S. Census, equating it to the distribution of number of children ever born to married native-born white women age 45–49 years in the North Central states, omitting women having no children. Women having 10 or more children, who were 2.9 per cent of all women, are pooled in the census so it was necessary to guess at appropriate numbers of $s = 10$ and above, arbitrarily ending at 15. By assigning the reasonable value of 2.000 to $\bar{B}$, B_s can then be calculated and then $\bar{B}'$. These values and calculations are given in the appendix table. $\bar{s}_i$ is found to be

APPENDIX TABLE. DATA FOR ESTIMATING BIAS IN THE ESTIMATE OF MEAN FERTILITY WHEN THE PROBABILITY OF ASCERTAINMENT IS PROPORTIONAL TO SIBSHIP SIZE. A REGRESSION OF FERTILITY ON SIBSHIP SIZE OF +0.1 AND A MEAN POPULATION FERTILITY OF 2.000 LIVEBIRTHS EVER BORN ARE ASSUMED. SEE TEXT FOR SYMBOLS AND EXPLANATION

s	θ_s	B_s
1	.2028	1.5890
2	.2445	1.6890
3	.1831	1.7890
4	.1244	1.8890
5	.0797	1.9890
6	.0571	2.0890
7	.0347	2.1890
8	.0279	2.2890
9	.0167	2.3890
10	.0110	2.4890
11	.0074	2.5890
12	.0046	2.6890
13	.0028	2.7890
14	.0018	2.8890
15	.0014	2.9890
Σ	.9999	—

$\bar{s}_i$ = 5.1102.
$\bar{B}$ = 2.001 (calculated from these data as a check).
$\bar{B}'$ = 2.176.

5.1102 ($\bar{s}$ = 3.4254), $\bar{B}$ calculated from the second equation above, as a check, is 2.001, but $\bar{B}'$ is 2.176. There is thus a positive bias of about 0.176 in the sample estimate of the mean fertility of normal individuals. This bias is seen in perspective when it is noted that the variance in the mean number of livebirths ever born to the normal sibs of sporadic propositi in the data of Crowe *et al.*, age 40 or more or deceased at any age, is 6.1495. The sample size of these sibs having a standard error of 0.176 is 198.5. Therefore, for sample sizes less than about 200 the bias is less than the standard error and, without appreciable error, may be neglected. For large samples collected through single ascertainment it would probably be worthwhile to calculate the expected bias and subtract it from the observed mean.

REFERENCES

Crowe, F. W., Schull, W. J., & Neel, J. V. 1956. *A Clinical, Pathological, and Genetic Study of Multiple Neurofibromatosis*. Springfield: C. C Thomas.

Fisher, R. A. 1950. *The Genetic Theory of Natural Selection*. Oxford: Oxford University Press.

Fisher, R. A. (Editor) 1935. The inheritance of fertility. Dr. Wagner-Manslau's tables. *Ann. Eugen.*, 6: 225–251.

Greenwood, M. & Yule, G. V. 1914. On the determination of size of family and of the distribution of characters in order of birth. *J. R. Statist. Soc.* 77: 179.

Haldane, J. B. S. 1935. The rate of spontaneous mutation of a human gene. *J. Genet.* 31: 317–326.

Krooth, R. S. 1955. The use of the fertilities of affected individuals and their unaffected sibs in the estimation of fitness. *Am. J. Human Genet.* 7: 325–360.

Pearson, K. P., Lee, A., & Bramley-Moore, L. 1899. Mathematical studies in evolution. VI. Genetic (reproductive) selection. *Phil. Trans. Roy. Soc. A.* 192: 257–330.

Penrose, L. S. 1949. The meaning of 'fitness' in human populations. *Ann. Eugen.*, 14: 301–304.

Penrose, L. S. 1950. Propagation of the unfit. *Lancet* ii: 425–427.

Reed, T. E. & Chandler, J. H. 1958. Huntington's chorea in Michigan. 1. Demography and genetics. *Am. J. Human Genet.* 10: 201–225.

Reed, T. E. & Neel, J. V. 1955. A genetic study of multiple polyposis of the colon (with an appendix deriving a method of estimating relative fitness). *Am. J. Human Genet.* 7: 236–263.

Reed, T. E. & Neel, J. V. 1959. Huntington's chorea in Michigan. 2. Selection and mutation. *Am. J. Human Genet.* 11: 107–136.

Slatis, H. M. 1955. Comments on the rate of mutation to chondrodystrophy in man. *Am. J. Human Genet.* 7: 76–79.

Weinberg, W. 1913. Zur Frage der Messung der Fruchtbarkeit. *Arch. Rass. -u. Ges. Biol.* 10: 162–166.

Whelpton, P. K. 1954. *Cohort Fertility*. Princeton: Princeton University Press.

Genetic Studies on Vitamin D Resistant Rickets (Familial Hypophosphatemia)

DAVID YI-YUNG HSIA, MICHAEL KRAUS, AND JUNE SAMUELS

Genetic Clinic, Children's Memorial Hospital, and Department of Pediatrics, Northwestern University Medical School, Chicago

RESISTANT RICKETS is a term used to described a clinical condition which is indistinguishable from ordinary rickets except that the signs occur in spite of an adequate intake of vitamin D in the diet. The disease is believed to occur because of a defect in the renal transport mechanism for phosphorus.

While ordinary rickets is uncommon in patients beyond the eighteenth month, refractory rickets persists or may even manifest itself for the first time in childhood. The clinical findings are those of florid rickets with bossing of the frontal bones, beading of the ribs, narrow flaring chest, prominent abdomen, swelling of the epiphyses of the wrists and ankles, and bowing of the legs and pronation of the feet. Progression of the defect in mineralization leads to retarded growth and skeletal deformities. Adults with this condition are usually in good general health, but may suffer from bone pains, especially in the back region.

Resistant rickets appears to occur because of a congenital failure of the renal tubules to reabsorb phosphorus. This results in excessive urinary loss of phosphorus and the plasma phosphate concentration remains at a permanently low level, usually in the order of 2 mgm per cent. This in turn leads to a failure of normal bone development which manifests itself with most of the classical features of rickets. Average doses of vitamin D have no effect upon the disease process, but the regular administration of massive amounts of the material will sometimes result in healing of the bone lesions. The condition has also been shown to be reversible when phosphorus is administered either intravenously or by mouth (Fraser, Geiger, Munn, Slater, Jahn, and Liu 1958).

The familial nature of the defect has been reported by a number of workers (Liebe 1939; Christensen 1940–41; Mackay and May 1945; Holt 1950; Imerslund 1951; Pedersen and McCarroll 1950; Coleman and Fotte 1954; Dent and Harris 1956; Tobler, Prader, and Taillard 1956; Swoboda 1956; Peterson 1956; Mitchell and Mitchell 1957; Winters, Graham, Williams, McFalls, and Burnett 1957 and 1958; Christiansson 1958; and Linneroth, Hallgren, Palmen, and Zetterström 1958). In most instances, the criteria for scoring have been primarily clinical and based upon typical histories and physical findings. Dent and Harris (1956) have suggested that the familial configuration is consistent with the hypothesis that the disorder is due to a rare abnormal dominant gene and that the affected individuals are heterozygous for this. In some instances, both of the parents of affected children have been found

These studies were aided in part by grants from the Otho S. A. Sprague Memorial Institute, and the Chicago Community Trust.

Received July 21, 1958.

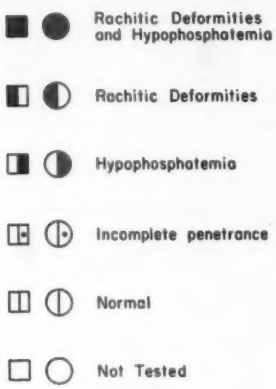
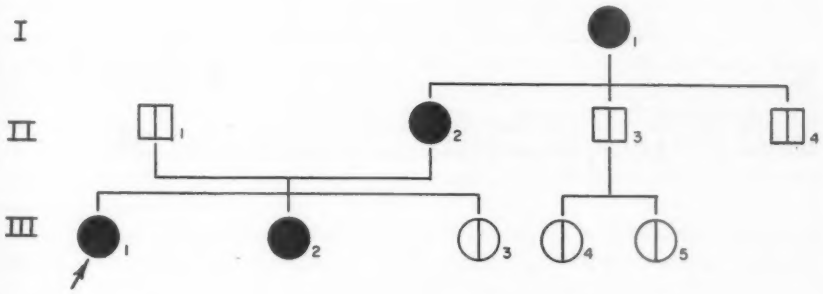
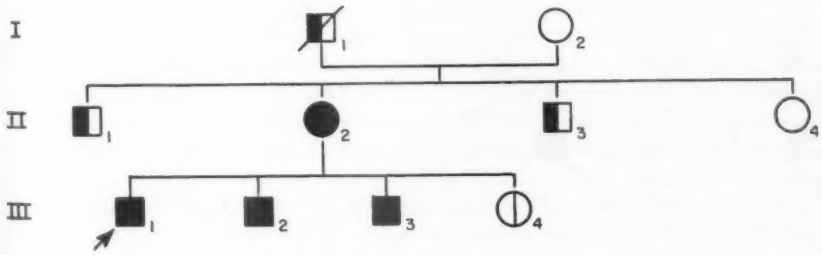


FIG. 1



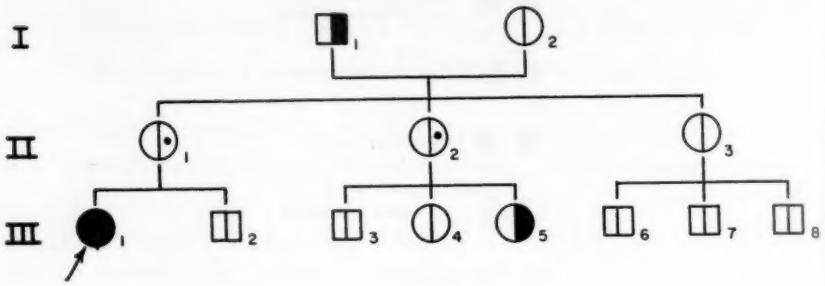
PEDIGREE Fu

FIG. 1a



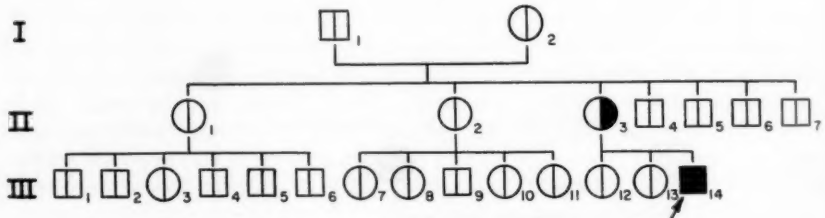
Sa

FIG. 1b



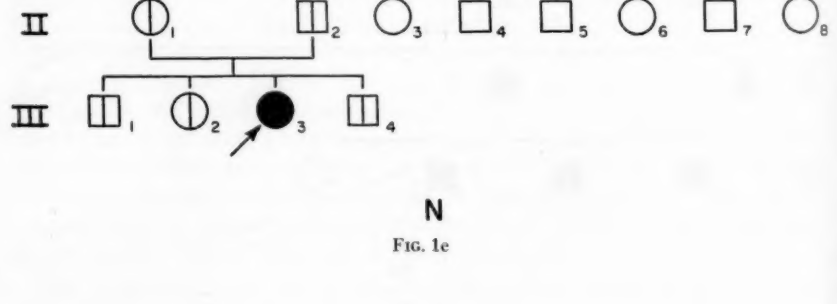
Fl

FIG. 1c



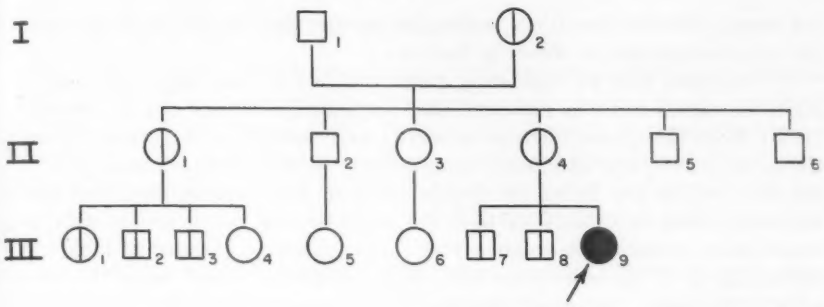
Sn

FIG. 1d



N

FIG. 1e



V

FIG. 1f

to be completely normal from the clinical viewpoint. Dent and Harris (1956) have suggested that the gene may be incomplete in its manifestation or that some of the sporadic cases may represent fresh mutations.

Recently, Winters and his coworkers (1957 and 1958) have reported on a large North Carolina kindred with this defect. They found that no clear hereditary pattern emerged if they used the presence of rachitic or past rachitic skeletal deformities for scoring affected individuals. However, when individuals were classified according to the level of serum phosphorus, hypophosphatemia was found to follow a sex-linked dominant pattern with complete penetrance.

The purpose of the present paper is to test this hypothesis by presenting the clinical, biochemical, and genetic findings on six additional families with resistant rickets. As far as is known, none of the pedigrees have been previously reported.

MATERIALS AND METHODS

All of the probands were originally seen in the Genetic Clinic of the Children's Memorial Hospital. In each instance, the diagnosis was established by clinical, radiological, and biochemical evidence of rickets and the failure of the children to respond to the usual prophylactic doses of vitamin D (500 International Units daily).

Each of the available relatives was examined clinically for bowing of the legs and other bony deformities. Wrists and knees were examined radiologically, and the films were reviewed by the same radiologist. In each instance, a sample of blood was taken for the determination of total serum calcium, inorganic phosphorus, and alkaline phosphatase. While no attempt was made to obtain fasting specimens, all were taken at least four hours after the previous meal. All of the biochemical determinations were done in the same laboratory under uniform conditions. The samples obtained locally were tested immediately, and those obtained from other cities were shipped by air in dry ice and tested within 24 hours after receipt. The serum calcium was determined by the method of Kramer and Tisdall (1921), and the inorganic phosphorus and alkaline phosphatase by the methods of Gomori (1941).

The sera from a group of 75 normal adults and 55 normal children were tested under the same conditions and served as controls. There were no differences between

the means, standard deviations, and ranges for the sexes in our sample and hence the data were grouped as shown in Appendix I.

It is apparent that we could have scored the data in three ways: (1) using the regression curves based on published data prepared by Winters and his coworkers (1958). While this approach obviously offers a larger sample one is hesitant to accept the values from several laboratories using different methods and personnel; (2) Using our own controls and basing the discrimination on 2½ standard deviations below the mean; (while on a theoretical basis this would be ideal, on the present data, this would place several of the children with clinical rickets in the normal range). (3) Classifying all of the individuals with serum phosphorus values below the lowest value of the normal controls as hypophosphatemic. Because of the obvious advantage of the third approach, all of the pedigrees have been scored in that manner.

RESULTS

The data on the six pedigrees are summarized in Figure 1 and in Appendix II.

Rickets or hypophosphatemia appears to have been transmitted through three generations in three of the families. In Pedigree Fu, clinical rickets as well as hypophosphatemia was transmitted from I-1 to II-2 to III-1 and III-2. In Pedigree Sa, a history of short stature and bow legs was reported for I-1, II-1, and II-3, and clinical rickets and hypophosphatemia were noted in II-2, III-1, III-2, and III-3. It is unfortunate that the grandfather (I-1) is dead, and that his two living male offspring live in Puerto Rico and hence are unavailable for testing. Otherwise, this would have been one of the very few examples of a male to male transmission of resistant rickets, the only other pedigrees having been reported by Liebe (1939) and Linneroth, Hallgren, Palmén, and Zetterström (1958). Even in the latter, the transmission is not established beyond doubt because of consanguinity and uncertainty of diagnosis on the paternal side.

In Pedigree Fl, hypophosphatemia was found in I-1. This grandfather was noted to have a serum phosphorus of 2.2 mgm per cent although he showed no clinical evidence of rickets. It is of interest to note that all three of his daughters (II-1, II-2, and II-3) showed no evidence of rickets and had essentially normal serum phosphorus levels. The conditions, however, reappeared in the third generation with III-1 showing clinical rickets and III-3 showing hypophosphatemia. It would appear that the defect had "skipped" the second generation and that penetrance is incomplete.

In three other families, information as to the mode of transmission is less satisfactory. In Pedigree Sn, borderline hypophosphatemia was found in the mother (II-3). Her child (III-14) showed clinical evidence of rickets, but the serum phosphorus levels were in the normal range because of vitamin D therapy. Neither of the maternal grandparents (I-1 and I-2) showed evidence of this trait. In Pedigree N, both parents were healthy and had normal serum phosphorus values and all of the relatives on the father's side appear by history to be free of rickets. In Pedigree V, only the mother and her family were available for testing. However, there is no evidence of clinical rickets on the father's side of the family by history.

DISCUSSION

The study of these six additional families with resistant rickets by biochemical as well as clinical means is useful for several reasons. In the first place, it confirms the findings of Winters *et al.* (1958) that the measurement of serum phosphorus levels is a more sensitive discriminant for this disorder than the use of clinical criteria alone. In Pedigree Sn, without biochemical studies, the mother (II-3), and in Pedigree Fl, the grandfather (I-1) and first cousin (III-5) of the proband would have been misclassified.

On the other hand, it would appear that despite the measurement of serum phosphorus levels, hypophosphatemia is not always inherited as a dominant characteristic with full penetrance. This is shown by the "skipping" in Pedigree Fl in two members of the second generation (II-1 and II-2). Also, in Pedigree N, both parents (II-1 and II-2) of the affected child (III-3), and in Pedigree Sn, both parents (I-1 and I-2) of the hypophosphatemic mother (II-3) failed to show significant alterations of serum phosphorus levels. While these may well represent fresh mutations, or an autosomal recessive form of the disease, the possibility of incomplete penetrance in the transmission of the trait must also be considered. It could be that complete penetrance is characteristic of the kindred studied by Winters *et al.* (1958), but that this may not apply to all instances of hypophosphatemia.

Finally, the information derived from the present families is not inconsistent with the hypothesis that hypophosphatemia is transmitted as a sex-linked dominant.

It would appear that until a properly documented instance of male to male transmission is reported, one would have to continue to accept the hypothesis that hypophosphatemia is transmitted as a sex-linked dominant. However, the families reported by Liebe (1939), Linneroth and his coworkers (1958), and the Pedigree Sa described here provide suggestive evidence that there may be more than one mode of inheritance for this disorder. If this is the case, the trait is transmitted as a sex-linked dominant in the majority of affected patients, and may act as an autosomal dominant or recessive in an occasional instance. In any event, fresh mutations may sometimes occur, and penetrance may be incomplete.

SUMMARY

Data have been presented from six additional families with vitamin D resistant rickets (familial hypophosphatemia). In three of the families, rickets or hypophosphatemia appears to have been transmitted through three generations. In one other, the mother shows a low serum phosphorus level. In the remaining two families, there are no other known instances of rickets among the parents or near relatives.

The data confirm the findings of Winters and his coworkers (1958) that the measurement of serum phosphorus levels offers a more sensitive discriminant for this disorder than the use of clinical criteria alone. However, even with the use of this approach, not all of the carriers are detectable. Furthermore, the possibility of fresh mutations have to be considered. While the family data support the hypothesis that hypophosphatemia is inherited as a sex-linked dominant in the majority of

instances, the possibility of an autosomal dominant or recessive mode of inheritance cannot be completely ruled out.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. H. Harris for helpful suggestions, to Dr. Harvey White for reviewing the films, and to Miss Grace Lawrence and Miss Margaret Reid for the Biochemical determinations.

REFERENCES

CHRISTENSEN, J. E. 1941. Three familial cases of atypical rickets. *Acta Paed.* 28: 247–270.

CHRISTIANSSON, G. 1958. Emergence of primary vitamin D resistance at puberty. Genetic study of primary rickets with faulty disposition. *Acta Paed.* 47: 288–296.

COLEMAN, E. N., AND FOOTE, J. B. 1954. Craniostenosis with familial vitamin D resistant rickets. *Brit. M. J.* 1: 561–562.

DENT, C. E. AND HARRIS, H. 1956. Hereditary form of rickets and osteomalacia. *J. Bone Surg.* 38B: 204–226.

FRASER, D., GEIGER, D. W., MUNN, J. C., SLATER, P. E., JAHN, R. AND LIU, E. 1958. Calcification studies in clinical vitamin D deficiency, and in hypophosphatemic vitamin D refractory rickets. *Am. J. Dis. Child.* 69: 460.

GOMORI, G. 1941. A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. *J. Laborat. Clin. M.* 27: 955–965.

HOLT, J. F. 1950. Vitamin D resistant rickets (refractory rickets). *Am. J. Roentg.* 64: 590–602.

IMERSLUND, O. 1951. Craniostenosis and vitamin D resistant rickets. *Acta Paed.* 40: 449–456.

KRAMER, B. AND TISDALL, F. F. 1921. A simple technique for the determination of calcium and magnesium in small amounts of serum. *J. Biol. Chem.* 47: 475–481.

LIEBE, S. 1939. Uber nicht heilbare Rachitis. *Monatschr. f. kinderh.* 78: 221–226.

LINNEROTH, K., HALLGREN, B., PALMEN, K., AND ZETTERSTROM, K. 1958. Primary (genuine) vitamin D resistant rickets. IV. A clinical and genetic study. *Acta Paed.* 47: 568–592.

MACKAY, H. AND MAY, Q. I. 1945. Rickets resistant to vitamin D, healing with very heavy dosage of vitamin D, fluctuations with vitamin D requirement, development of hypercalcemia. *Proc. R. Soc. M.* 38: 565.

MITCHELL, F. N., AND MITCHELL, J. E. 1957. Vitamin D resistant rickets. *Am. J. Dis. Child.* 93: 385–390.

PEDERSEN, H. E. AND MCCARROLL, H. R. 1951. Vitamin-resistant rickets. *J. Bone Surg.* 38A: 203–220.

PETERSON, R. E. 1956. Hypophosphatemic rickets. Description and case reports of renal tubular form of this deficiency disease. *J. Kansas M. Soc.* 57: 582.

SWOBODA, W. 1956. Die genuine Vitamin D resistante Rachitis *Wien. Beit. Kinderh.* 6: 1–79.

TOBLER, R., PRADER, A., AND TAILLARD, W. 1956. Die familare vitamin D resistante Rachitis (Phosphatdiabetes). *Helvet. Paediat. Acta* 11: 209–255.

WINTERS, R. W., GRAHAM, J. B., WILLIAMS, T. F., MCFALLS, V. W., AND BURNETT, C. H. 1957. A genetic study of familial hypophosphatemia and vitamin D resistant rickets. *Tr. Ass. Am. Physicians* 52: 234–242.

WINTERS, R. W., GRAHAM, J. B., WILLIAMS, T. F., MCFALLS, V. W. AND BURNETT, C. H. 1958. A genetic study of familial hypophosphatemia and vitamin D resistant rickets with a review of the literature. *Medicine* 37: 97–142.

APPENDIX I. VALUES OF NORMAL CONTROLS

		Serum Calcium	Serum Phosphorus	Serum Alkaline Phosphatase
Adults	No.	73	75	44
	Mean	10.3 mgm%	3.3 mgm%	3.7 B.U.*
	S.D.	±0.9	±0.4	±1.4
	Range	8.8–13.8	2.4–5.3	1.1–8.7
Children	No.	62	55	54
	Mean	10.9 mgm%	4.9 mgm%	8.2 B.U.
	S.D.	±1.3	±0.8	±2.7
	Range	8.5–12.5	3.2–6.7	2.5–14.7

* Bodansky units

APPENDIX II. SUMMARY OF DATA ON FAMILIES

	Living	Sex	Parents	Examined	Year of Birth	Clinical Rickets*	X-ray Rickets	Calcium (mgm%)	Phosphorus (mgm%)	Phosphatase (B.U.)
Pedigree Fu										
I-1	L	F	—	E	1918	*Yes*	Neg.	11.7	*1.8*	5.2
II-1	L	M	—	E	1929	No	—	10.5	3.8	2.2
II-2	L	F	I-1	E	1934	*Yes*	*Pos.*	9.1	*2.1*	6.4
II-3	L	M	I-1	E	1932	No	Neg.	11.9	3.5	4.1
II-4	L	M	I-1	E	1930	No	Neg.	—	—	—
III-1	L	F	II-2	E	1952	*Yes*	*Pos.*	10.8	*2.9*	6.6
III-2	L	F	II-2	E	1954	*Yes*	*Pos.*	9.7	*2.0*	5.9
III-3	L	F	II-2	E	1956	No	Neg.	10.0	6.4	5.8
III-4	L	F	II-3	E	1951	No	Neg.	12.1	4.6	9.5
III-5	L	F	II-3	E	1953	No	Neg.	11.5	5.4	8.3
Pedigree Sa										
I-1	D	M	—	Ne	—	*Yes*	—	—	—	—
I-2	D	F	—	Ne	—	No	—	—	—	—
II-1	L	M	I-1 & 2	Ne	1919	*Yes*	—	—	—	—
II-2	L	F	I-1 & 2	E	1921	*Yes*	—	—	*2.2*	7.8
II-3	L	M	I-1 & 2	Ne	1932	*Yes*	—	—	—	—
II-4	L	F	I-1 & 2	Ne	1934	No	—	—	—	—
III-1	L	M	II-2	E	1952	*Yes*	*Pos.*	11.3	*2.4*	17.6
III-2	L	M	II-2	E	1953	*Yes*	*Pos.*	11.8	*2.4*	16.8
III-3	L	M	II-2	E	1954	*Yes*	*Pos.*	9.6	*2.3*	12.1
III-4	L	F	II-2	E	1957	No	Neg.	11.3	5.6	8.8

APPENDIX II—*Continued*

	Living	Sex	Parents	Examined	Year of Birth	Clinical Rickets*	X-ray Rickets	Calcium (mgm%)	Phosphorus (mgm%)	Phosphatase (B.U.)
					Pedigree Fl					
I-1	L	M	—	E	1906	No	Neg.	9.6	*2.2*	3.8
I-2	L	F	—	E	1909	No	Neg.	10.2	3.1	2.8
II-1‡	L	F	I-1 & 2	E	1926	No	Neg.	9.6	3.3	4.9
II-2‡	L	F	I-1 & 2	E	1928	No	Neg.	9.7	3.0	2.6
II-3	L	F	I-1 & 2	E	1931	No	Neg.	10.4	3.05	3.3
III-1	L	F	II-1	E	1951	*Yes*	*Pos.*	11.6	*2.4*	—
III-2	L	M	II-1	E	1957	No	Neg.	10.7	4.5	—
III-3	L	M	II-2	E	1947	No	Neg.	9.3	4.8	8.2
III-4	L	F	II-2	E	1948	No	Neg.	9.8	4.2	10.6
III-5	L	F	II-2	E	1952	No	Neg.	9.7	*3.0*	2.6
III-6	L	M	II-3	E	1951	No	Neg.	10.8	4.9	6.8
III-7	L	M	II-3	E	1954	No	Neg.	10.2	4.5	10.6
III-8	L	M	II-3	E	1957	No	Neg.	11.4	7.4	7.4
					Pedigree Sn					
I-1	L	M	—	E	1896	No	Neg.	9.9	2.6	1.9
I-2	L	F	—	E	1912	No	Neg.	11.2	3.2	3.0
II-1	L	F	I-1 & 2	E	1924	No	Neg.	9.5	4.0	1.1
II-2	L	F	I-1 & 2	E	1926	No	Neg.	10.8	3.1	3.8
II-3	L	F	I-1 & 2	E	1927	No	Neg.	11.6	*2.4*	2.4
II-4	L	M	I-1 & 2	E	1936	No	Neg.	10.3	3.9	2.2
II-5	L	M	I-1 & 2	E	1939	—	—	—	—	—
II-6	L	M	I-1 & 2	E	1942	No	Neg.	11.2	5.1	9.2
II-7	L	M	I-1 & 2	E	1945	No	Neg.	10.3	4.0	4.5
III-1	L	M	II-1	E	1951	No	Neg.	9.5	5.2	7.0
III-2	L	M	II-1	E	1945	No	Neg.	9.9	4.1	2.4
III-3	L	F	II-1	E	1952	No	Neg.	9.9	4.5	4.5
III-4	L	M	II-1	E	1955	No	Neg.	9.5	4.7	5.8
III-5	L	M	II-1	E	1954	No	Neg.	9.5	6.4	5.3
III-6	L	M	II-1	E	1955	No	Neg.	9.9	5.6	5.5
III-7	L	F	II-2	E	1945	No	Neg.	10.3	4.7	7.1
III-8	L	F	II-2	E	1947	No	Neg.	10.3	6.2	5.8
III-9	L	M	II-2	E	1949	No	Neg.	10.3	5.9	4.9
III-10	L	F	II-2	E	1951	No	Neg.	11.2	5.8	9.8
III-11	L	F	II-2	E	1953	No	Neg.	10.8	5.6	6.8
III-12	L	F	II-3	E	1945	No	Neg.	10.8	4.0	6.4
III-13	L	F	II-3	E	1953	No	Neg.	10.8	4.4	3.6
III-14	L	M	II-3	E	1954	*Yes*	*Pos.*	8.6	*4.0*†	81.4

APPENDIX II—*Continued*

	Living	Sex	Parents	Examined	Year of Birth	Clinical Rickets*	X-ray Rickets	Calcium (mgm%)	Phosphorus (mgm%)	Phosphatase (B.U.)
					Pedigree N					
I-1	D	M	—	Ne	—	—	—	—	—	—
I-2	L	F	—	E	1915	No	—	10.2	3.0	2.8
II-1	L	F	—	E	1932	No	Neg.	10.5	4.5	3.1
II-2	L	M	I-1 & 2	E	1930	No	Neg.	11.3	3.2	3.2
II-3	L	F	I-1 & 2	E	1932	—	—	—	—	—
II-4	L	M	I-1 & 2	E	1937	—	—	—	—	—
II-5	L	M	I-1 & 2	E	1948	—	—	—	—	—
II-6	L	F	I-1 & 2	E	1950	—	—	—	—	—
II-7	L	M	I-1 & 2	E	1953	—	—	—	—	—
II-8	L	F	I-1 & 2	E	1956	—	—	—	—	—
III-1	L	M	II-1 & 2	E	1949	No	Neg.	12.1	5.5	10.5
III-2	L	F	II-1 & 2	E	1955	No	Neg.	12.1	5.0	11.0
III-3	L	F	II-1 & 2	E	1956	*Yes*	*Pos.*	8.1	*2.0*	47.6
III-4	L	M	II-1 & 2	E	1957	No	Neg.	11.3	5.6	10.4
					Pedigree V					
I-1	L	M	—	Ne	—	—	—	—	—	—
I-2	L	F	—	Ne	1913	No	Neg.	11.1	2.9	5.4
II-1	L	F	I-1 & 2	E	1933	No	Neg.	11.0	3.4	2.5
II-2	L	M	I-1 & 2	Ne	1934	—	—	—	—	—
II-3	L	F	I-1 & 2	Ne	1936	—	—	—	—	—
II-4	L	F	I-1 & 2	E	1938	No	Neg.	9.9	3.6	5.4
II-5	L	M	I-1 & 2	Ne	1942	—	—	—	—	—
II-6	L	M	I-1 & 2	Ne	1950	—	—	—	—	—
III-1	L	F	II-1	E	1948	No	Neg.	11.0	5.1	10.6
III-2	L	M	II-1	E	1951	No	Neg.	10.2	5.2	11.5
III-3	L	M	II-1	E	1953	No	Neg.	11.4	7.4	—
III-4	L	F	II-1	E	1954	No	—	—	—	—
III-5	L	F	II-2	Ne	1957	—	—	—	—	—
III-6	L	F	II-3	Ne	1952	—	—	—	—	—
III-7	L	M	II-4	E	1954	No	Neg.	11.2	5.6	13.6
III-8	L	M	II-4	E	1955	No	Neg.	11.2	6.4	17.6
III-9	L	F	II-4	E	1957	*Yes*	*Pos.*	10.4	*2.0*	97.2

* Clinical history of rickets which has been verified by direct examination in all families except Pedigree Sa.

† Sample taken while patient on vitamin D therapy.

‡ Presumed carrier of trait.

Dominant Ectopia Lentis et Pupillae

GORDON L. WALLS AND GORDON G. HEATH

School of Optometry, University of California, Berkeley and Division of Optometry, Indiana University, Bloomington

IN 1953 a 30-year-old man (II-6 in the accompanying chart) came into clinic with an asthenopic complaint, was noted to be color blind, and was referred to us primarily for color-vision testing. He proved to be a protanope and, his right eye being aphakic, he afforded an unusual opportunity to ascertain the effect of normal lens coloration upon the position of the protanopic spectral neutral point (he became Subject No. 44 in Walls and Heath, 1956).

The aphakia in this instance was surgical, necessitated by trauma from a dynamite blast suffered at age 22. Prior to that, II-6 had had normally centered lenses and pupils; but, he volunteered that he had sisters with congenitally eccentric lenses and no lenses at all. A survey of the family was commenced, which has now been carried as far as seems profitable.

II-1 and II-4 are now 37. They are totally unlike in habitus and can be assumed to be dizygous, although unfortunately the scientific criteria were not noted down at their (home) delivery. The pupils of II-1 have always been circular and centered, but from birth both lenses were eccentric. She had low visual acuity and a right esotropia up to age 11, when the right lens became luxated and was removed. Her right eye now had 20/50 vision—much better than the left—and she became a left esotrope. She saw either through or past her left lens depending upon the posture of her head. By age 22 this had become so annoying that she secured the removal of the left lens; thereafter the left eye enjoyed 20/40 vision.

Both lenses of II-4 were congenitally displaced upward and outward, as were also her pupils which are illustrated here. Her right lens has remained fixed in position, but for years she could look either through or past the left one at will, by tilting her head. Some months ago the left lens became luxated, and it has now "couched" itself.

In II-8 (now 30) both pupils are normal, but the left lens has always been displaced upward and inward. Excepting in very strong illumination, its margin shows in the pupil. II-8 can look either through the lens or past it; but this is a matter of attention—she does not need to tilt her head this way or that.

All of the children in generation III have been examined recently by able specialists: III-1 (17) and III-2 (11) by one of us (G. G. H), III-3 (12) and III-4 (6) by Dr. Harold F. Falls, and III-5 (7½), III-6 (5½), III-7 (3), and III-8 (2) by Dr. Henry A. Knoll. All have completely normal anterior segments excepting III-4, in whom the right lens is ectopic but not far enough so for its margin to present in the pupil. Both irides are tremulous, but both pupils are normal.

None of the marriages shown in the chart is known, nor can be suspected, to be consanguineous. With the defect in III-4 in essentially the same form as in one of

Received September 29, 1958.

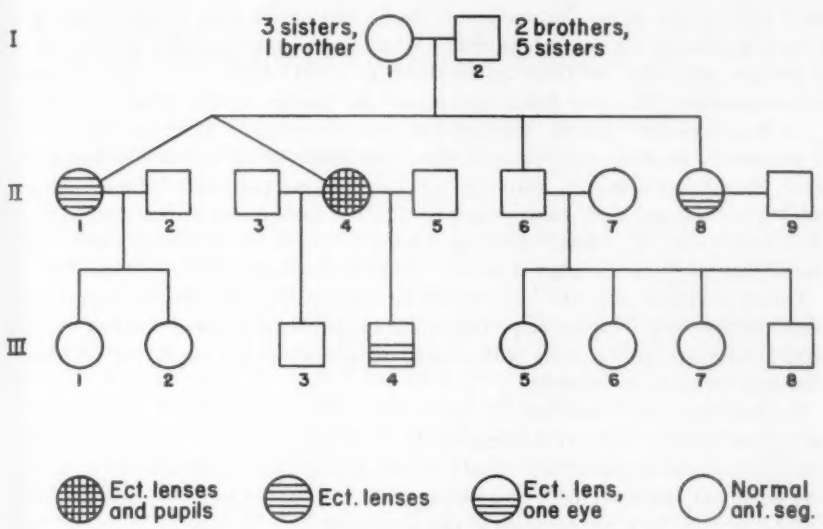


CHART 1

FIG. 1. Iris outlines of II-4, showing shapes, locations, and sizes of pupils as drawn in evening artificial illumination (kitchen).

his aunts (II-8), the gene concerned is clearly indicated to be a dominant with variable expression ranging from an ectopic lens in one eye and normal pupils to ectopic lenses and pupils in both eyes. There is no suggestion of arachnodactyly anywhere in the kindred. For the condition to be recessive (barring an unlikely change of dominance), I-1, I-2, and II-5 would have to be heterozygotes. II-5 knows of no such ophthalmic condition in any relative. I-1 has three sisters and one brother, all of whom, together with descendants to great-grandchildren, are reputed normal. I-2 has two brothers and five sisters, and no form of the defect is known for any of these or their descendants.

Wondering how uncommon such a situation might be, we described it in letters to four leading ophthalmic geneticists and asked for their comments. Helpful replies were received from all four—Drs. P. J. Waardenburg, A. Sorsby, A. Franceschetti, and H. F. Falls.

Waardenburg reiterated the conclusions he had reached earlier in his book (1932): ectopia lentis combined with ectopia pupillae is a single recessive character, but unstable in manifestation so that in the same sibship there may be individuals

with only ectopic lenses (in one eye or both) and others with ectopic lenses and ectopic pupils also (in one or both eyes). To earlier cases, he was able to add that of a brother and sister described by Diethelm in a 1947 thesis. Their parents were consanguineous. The sister had ectopic lenses; the brother, ectopic lenses and pupils.

In Waardenburg's opinion, when ectopia lentis really stands alone in a family and is not merely an under-expression, in some individuals, of an ectopic-lens-and-pupil gene, then it is a dominant character. So also when ectopia lentis (without ectopic pupils) is combined with arachnodactyly. The one pedigree of Strebel and Steiger, described in the 1932 book, exhibiting dominant ectopic-lens-and-pupil (with "predisposition to rheumatic heart defects") still had no known counterpart in 1958.

Sorsby could add nothing to what was in his own (1951) book, where his conclusions were identical with Waardenburg's (1932). He agreed that we were apparently dealing with a dominant gene causing both ectopic lenses and ectopic pupils, but with considerable variation in expression.

Franceschetti also pronounced it unusual for the combination of ectopic lenses and ectopic pupils to behave dominantly. He reminded us that we should exclude the possibility of consanguinity of I-1 and I-2, or of II-4 and II-5, before assuming a new and dominant mutation; and, he pointed out that we never could exclude the faint possibility that the gene operating in our generation II is the familiar recessive one, changing to a dominant one in III-4. While this last suggestion is radical in one sense, it is conservative in another, since it would keep our material under the banner of recessive ectopic-lens-and-pupil, and obviate postulation of a new mutation.

Falls was able to tell us that even recessive ectopia lentis et pupillae is not a single genetic entity, for in certain families the displacement of the pupils is always down and in, particularly when associated with extreme degrees of myopia, whereas in other families this is not so. He also pointed out that in ectopia lentis families "any abnormality of the anterior chamber might be anticipated". Here, Falls was alluding to "Axenfeld's syndrome", a dominant and highly variable dysgenesis mesodermalis or posterior embryotoxon in which the pupil and lens are occasionally involved, with either or both ectopic.

During a visit to the West Coast, Dr. Falls was kind enough to examine II-4 and her two sons. He confirmed the normality of III-3 and the presence in III-4 of dextrocular ectopia lentis and binocular iridodonesis. These examinations included biomicroscopy, but apparatus for gonioscopy was not available to Dr. Falls. He saw no evidence of Marfan's syndrome, and agreed that our family exhibits a dominant defect.

We conclude that in this family a "new" dominant gene of variable expressivity is involved. The dominant inheritance here of ectopia lentis et pupillae, isolated from Marfan's syndrome and unaccompanied by "predisposition to rheumatic heart defects," appears to be unique.

REFERENCES

SORSBY, A. 1951. *Genetics in Ophthalmology*, London: Butterworth.

WAARDENBURG, P. J. 1932. *Das menschliche Auge und seine Erbanlagen*, 's-Gravenhage: Martinus Nijhoff. (= Deel VII of *Bibliographica Genetica*, ed. J. P. Lotsy & W. A. Goddijn.)

WALLS, G. L. AND HEATH, G. G. 1956. Neutral points in 138 protanopes and deuteranopes. *J. Optic. Soc. America* 46: 640–649.

A Possible Genetic Basis for Kuru

J. H. BENNETT, F. A. RHODES, AND H. N. ROBSON

Departments of Genetics and of Medicine, University of Adelaide, South Australia

INTRODUCTION

WIDESPREAD INTEREST has been aroused by Zigas and Gajdusek's (1957) description of their extensive observations on kuru, a rapidly progressive neurological disorder found with a remarkably high frequency among the Fore natives of New Guinea. Clinically, kuru runs a fairly uniform course, the average duration being about nine months. The onset is with ataxia of the legs, without antecedent illness. This ataxia becomes progressively more marked and more generalised, the victim gradually becoming unable to walk and finally quite helpless. A fine generalised tremor, aggravated by effort or emotion is usually present, and gross involuntary movements are seen. The general picture is one of progressive disorder of cerebellar function. Psychic changes are also present in many cases, commonly taking the form of an euphoria (Simpson, Lander and Robson, 1958). The disease clinically resembles some types of cerebello-spinal degeneration, in many of which a familial incidence has been recorded (Greenfield, 1954). The pathological observations are consistent with abiotrophy or a toxic factor as causative agent (Fowler and Robertson, 1958). A great many environmental factors have been examined as possible causative agents but only negative results have been obtained (Zigas and Gajdusek, 1957). Further, it appears that a single environmental factor could scarcely account for some more recent observations on the occurrence of kuru (Gajdusek and Zigas, 1958).

In their first article Zigas and Gajdusek had already raised the possibility of the disease having a genetical basis. However, no pedigree study was described. One might well wonder how, if at all, suitable pedigrees could be obtained for Fore families. Even if native diagnosis and memory should prove satisfactory for this purpose, we should expect the genetical study of kuru to involve a number of difficulties in addition to those usually associated with the collection and analysis of human genetical data. First and foremost, the Fore are warlike primitive people living in a wild and very remote highland area. Further, the disorder can as yet only be diagnosed in a victim a few months before death. Many individuals dying of other causes might have developed kuru later in life. This must add to the complexities of the analysis.

Since it is only through the collection and analysis of pedigree material that the question of a possible genetical basis for kuru can be settled, a deliberate attempt was made during a recent visit to the Fore area to obtain a large body of suitable genealogical material. A preliminary account of some of our findings has been set down in an earlier article (Bennett, Rhodes and Robson, 1958). The present article gives a more complete description of the collection, analysis and interpretation of the genealogical data and of the implications for further genetical research into this puzzling and tragic disease.

Received October 23, 1958.

The Fore People

The Fore people are an isolated linguistic group of some 10,000 individuals inhabiting an area of about 500 square miles of rugged country to the east of Mt. Michael in the eastern highlands of New Guinea (see Fig. 1). As far as we know, there has been no major movement of the Fore outside the present tribal area in recent times. Gajdusek and Zigas (1958) have reported that some intermarriage has occurred in border regions between the Fore and the Kimi, Keigana, Kanite, Iate and Usurufa tribes but not with the Kukukuku, Awa or Auiana people, and further that kuru occurs only among the Fore and in those neighbouring groups with a tradition of intermarriage with them.

The Fore themselves believe that kuru is induced by sorcery and a well-defined and established ritual is recognised (Berndt, 1958). The close relatives of a kuru victim regard it as their duty to identify the individual thought to have been the sorcerer who may then be murdered according to the ritual known as "tokabu." The Fore are known and feared by neighbouring tribes over quite a large area on account of the powers of sorcery which they are believed to possess. The extent of the area over which this reputation has spread may be some indication that kuru has existed for a considerable time.

The Fore community is composed of very many small units or hamlets, comprising a few huts, commonly five or six, usually situated on the summit of a ridge. A number

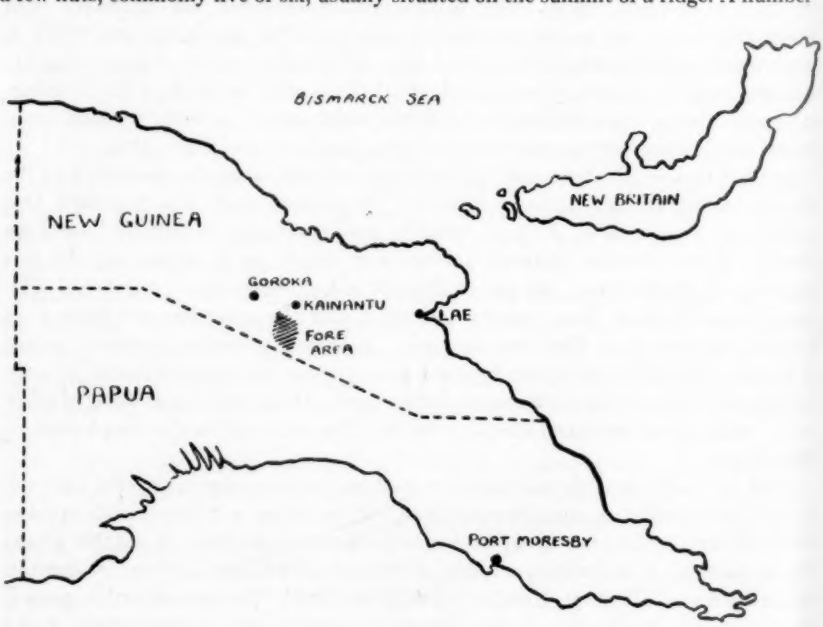


FIG. 1. Map of New Guinea, showing the Fore area.

of these hamlets, often scattered over quite a large area, together form a "village." The area thus occupied, the village ground, is clearly defined and remains fixed although the position of the hamlets within this area and the individual groupings of the huts may change frequently. The village is the political and social unit of society.

Until very recently the Fore people often indulged in small-scale warfare amongst themselves on an inter-village basis. Temporary alliances between two or more villages for this purpose occurred but it seems that these groupings changed frequently. The freedom of movement of an individual within the tribal area at any time would have depended on the political relations of the various villages at that moment, but provided he did not enter the ground of an enemy village, he could have travelled quite widely.

The Fore are polygamous. Females tend to be married at about 14 years of age and frequently to much older men. Unmarried men of up to 30 years of age are quite common. According to Berndt (1954) first cousin marriages are preferred, but the present authors have found little evidence of this. However, we did find that after the death of his wife a man commonly would marry one of her sisters, or alternatively, after the death of her husband a woman would join the household of one of his brothers. Divorces, when they occur, are usually agreed to on grounds of childlessness. Promiscuity is not evident.

When twins are produced, the second is killed at birth. When a young mother dies, her children are immediately adopted either by the other wives of the father or by close relatives. These children are then regarded as brothers and sisters of existing children of the adopting couple. However, the blood relationships are not forgotten.

Apart from kuru and tokabu, the commonest causes of death would appear to be respiratory infections, trauma and dysentery. Malaria occurs infrequently.

It is only in the last few years that the Fore have come into contact with Europeans. An Administration patrol post was established at Okapa in 1954 and the patrol officer and the medical assistant there and missionaries at Purosa and Anumpa are still the only Europeans who have lived permanently in the area. Dr. V. Zigas, the medical officer stationed at Kainantu was the first to call medical attention to kuru in 1955.

COLLECTION OF DATA

Most of the genealogical data on which this article is based were collected by two of us (F. A. R. and H. N. R.) during January 1958. Data were obtained in three different ways and recorded in three separate files:—

The Moke-Kagu or R-File

The genealogical data included in this file were collected from as many as possible of the inhabitants of a number of villages (shown on the accompanying map, Fig. 2) mostly situated along a track running northwards from Moke near the centre of the kuru region to Kagu near the Fore tribal boundary. Communication was achieved through a pidgin-speaking native. Families were ascertained without reference to kuru. The starting point was usually a group of older men and women, each of whom was questioned in turn. Genealogies were extended backwards as far as the memories

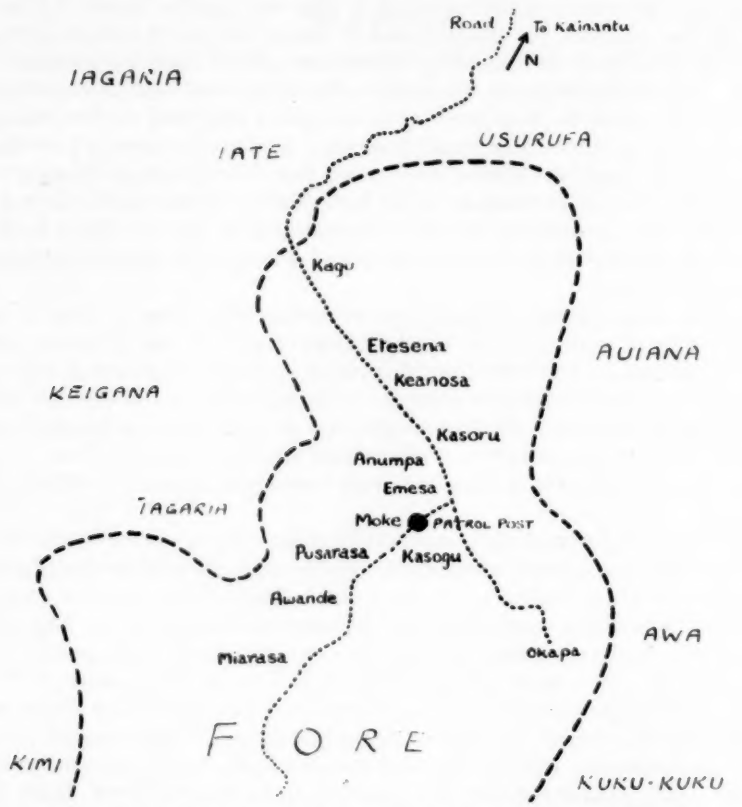


FIG. 2. Map of the northern part of the Fore area showing the location of the villages included in the Moke-Kagu (R-file) survey.

of our informants would permit. In each instance, the name, sex and birth order were recorded. For those thought to be under 17 years of age, the approximate age was also recorded. For deceased individuals, age at death was estimated by an indication of height in the case of children and by similarity to known living individuals in the case of adults. However, no estimate of the age at death was recorded (i) whenever this was thought to have been 17 years or more, or (ii) for any mother. The cause of death was asked for and this was recorded as kuru, tokabu, other given cause or cause unknown. Fortunately for our purpose, kuru with its distinctive clinical picture is readily recognised by the Fore people.

Repeated visits to the same villages to obtain further details and the independent questioning of different members of the same family enabled much cross-checking of information to be carried out. We were very impressed by the consistency of the information supplied.

The K-File

This file contains the pedigrees of 10 families each of which was ascertained through a kuru victim seen by one of us (H. N. R.) in January 1958.

The Z-File

The contents of this file were most generously made available to us by Dr. V. Zigas. It includes pedigrees of 62 families each of which was ascertained through a kuru victim examined by Zigas or Gajdusek. Most of these victims came from parts of the Fore area not covered by the R-file.

In all cases where an individual appeared in more than one pedigree, appropriate cross references were entered in the records. There was no overlap between the K- and Z-files and very little overlap between the R-file on the one hand and the K- and Z-files on the other hand. Four of the 181 pedigrees in the R-file were connected through kuru victims with four of those in the Z-file, and another three were connected with three in the K-file. For purposes of pedigree analysis, the few individuals recorded in more than one file have been counted in the R-file only.

RESULTS

Sex and Age Distribution

The R-file contains 181 pedigrees with records of 2099 individuals as shown in Table 1 A. The frequencies for the separate villages included in the survey are shown in Table 1 B. The over-all sex-ratio, 110 males: 100 females, shows a surprisingly large proportion of males. It may be that if the Fore practice infanticide this discriminates more often than not against females but we have not been able to gather reliable information on this point. There is an even greater excess of males among those individuals alive at the time of study, as many as 670 out of 1186, or 56.5 per cent, being of this sex. The added excess is probably a reflection of the effects of kuru which was responsible for the deaths of 177 females but only 27 males. On the other hand, 373 males died of tokabu or other known causes as against only 262 females.

TABLE 1. CLASSIFICATION OF INDIVIDUALS RECORDED IN THE R-FILE
A. TOTALS

Sex	Age	Alive	Dead				Totals
			Kuru	Tokabu	Other cause	Cause unknown	
F	Adult	312*	156	34	171	36	709
	Young	204	21	3	54	4	286
	Total	516	177	37	225	40	995
M	Adult	440	8	47	248	32	775
	Young	230	19	4	74	2	329
	Total	670	27	51	322	34	1104
Grand Totals		1186	204	88	547	74	2099

* Including one "recovered kuru."

Note: Throughout the tables, "adult" age is taken to be 17 years or more.

TABLE 1. B. FREQUENCIES FOR THE SEPARATE VILLAGES IN THE R-FILE

Villages	Age	Females					Males					Total
		Alive	Dead				Alive	Dead				
			Kuru	Tokabu	Other cause	Cause unknown		Kuru	Tokabu	Other cause	Cause unknown	
Kagu	A	19	19	2	10	2	26		5	15		98
	Y	17	1	1	7		14		1	7		48
Etesena	A	59	29	8	30	7	85	2	13	55	8	296
	Y	43	6		20	2	49	4	2	15	1	142
Keanosa	A	43	11	7	21	3	55	1	10	27	2	180
	Y	27	1	1			25	2		1		57
Kasoru	A	8	4	2	3	4	11		2	14	1	49
	Y	5			2		11		1	2		21
Anumpa	A	18	4		6		14			7	1	50
	Y	13	1				11			1		26
Emesa	A	3	1	1	2		5					12
	Y	2			1		3					6
Moke	A	120	67	11	80	3	186	4	15	108	5	599
	Y	76	9		21	1	89	8		44		248
Kasogu	A	23*	7	3	17	2	29		2	21		104
	Y	8	1	1	3		9	2		3		27
Awande	A	8	6			11	15				9	49
	Y	8					11					19
Miarasa	A	11	8		2	4	14	1		1	6	47
	Y	5	2			1	8	3		1	1	21

* Including one "recovered kuru."

As has already been mentioned, estimates of the ages of those who died from kuru in childhood were obtained when the data of the R-file were collected. Subsequently, rough age estimates of those who had died of kuru later in life were set down in 10-year groups. This was done by taking account of birth order, the number of offspring, and any other relevant information included in their pedigrees. The histograms for these data are given in Fig. 3.

Zigas and Gajdusek (1957) have described the age distribution in 154 kuru victims whom they had seen and studied. They have very kindly made available to us their estimates of age for 193 kuru victims (including the 154 already mentioned). The histograms shown in Fig. 4 are obtained when five-year class intervals are used to group these data. There is a remarkably close similarity between these age distribu-

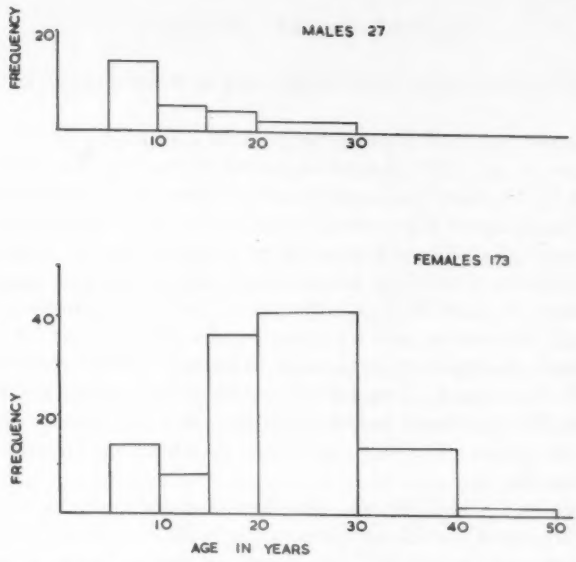


FIG. 3. Histograms showing distribution of estimated age at death of kuru victims included in the R-file.

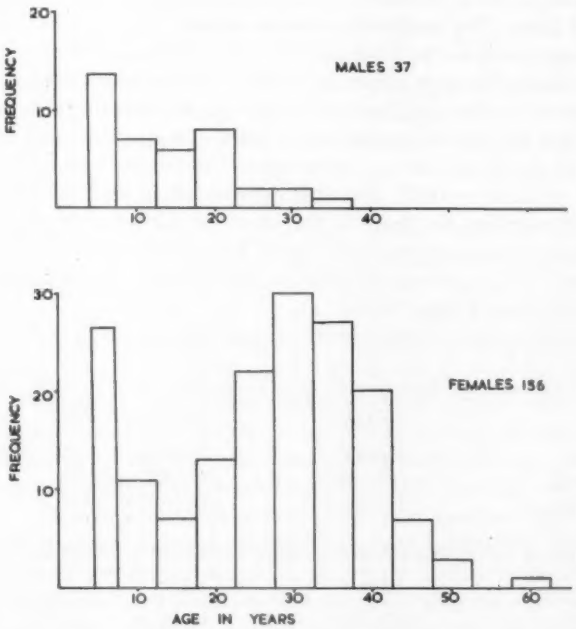


FIG. 4. Histograms showing distribution of age in 193 kuru victims seen by Zigas and Gajdusek (1958).

tions and those of kuru victims in the R-file many of whom died one or two generations ago.

It is to be noted that there is as yet no record of kuru having occurred in a child under four years of age. The youngest victims were two boys aged four and a girl aged five, seen by Zigas and Gajdusek. In males, where the distribution seems to be unimodal, the mean age of kuru victims is about 14 years. In females, on the other hand, many more cases of kuru have occurred in adults than in children. The age distribution in females is definitely bimodal, one mode occurring at about eight and the other at about 33 years. We may look upon this bimodal distribution as the sum of two unimodal distributions, one for young females and the other for adults. It is therefore useful to distinguish between cases of kuru in females with "early-onset" and those with "later-onset," even though no clinical or histological difference has yet been found. For our present purposes, we have chosen 17 years as the boundary between the two groups. This age comes close to separating the two component distributions (see Fig. 4).

Table 2 shows the 2 x 2 classification according to sex and young (or "early-onset") *vs* adult (or "late-onset") of all members of the R-file said to have died of kuru. The same classification for the 193 kuru victims studied by Zigas and Gajdusek is given in Table 3. The latter shows a higher proportion of males amongst the kuru victims than does the R-file, but the difference is not significant at the 0.05 level of probability. Table 4 shows the same 2 x 2 classification for all kuru victims included in the K- and Z-files. The proportion of males amongst the victims in the Z-file is significantly higher than in the R-file ($\chi_1^2 = 5.5$, $P = 0.02$). As we shall see later, the Z-file contains an unduly high proportion of families ascertained through males and this could account for the significant difference just mentioned. There is also a significant difference between the proportions of female cases of kuru said to have been of late-onset in the R-file and in the group of 193 victims studied by Zigas and Gajdusek ($\chi_1^2 = 5.2$, $P = 0.02$). The higher proportion in the R-file may be due to our practice of recording the death of any ancestral relative as having occurred in adulthood unless it was known to be otherwise. Thus, whilst the Z-file certainly gives a better indication of the true age distribution, the R-file may give a better indication of the sex distribution of kuru victims.

It should be noted that the total number of kuru cases found in males is very close

TABLE 2. SEX AND AGE DISTRIBUTION OF 204 REPORTED CASES OF KURU (R-FILE)

	Females	Males	Total
Adult	156 (77%)	8 (4%)	164
Young	21 (10%)	19 (9%)	40
Total	177 (87%)	27 (13%)	204

TABLE 3. SEX AND AGE DISTRIBUTION OF 193 OBSERVED CASES OF KURU (ZIGAS & GAJDUSEK, 1958)

	Females	Males	Total
Adult	122 (63%)	16 (8%)	138
Young	34 (18%)	21 (11%)	55
Total	156 (81%)	37 (19%)	193

TABLE 4. SEX AND AGE DISTRIBUTION OF ALL CASES OF KURU INCLUDED IN THE K- AND Z-FILES

	Females	Males	Total
K-File			
Adult	12 (60%)	1 (5%)	13
Young	4 (20%)	3 (15%)	7
Total	16 (80%)	4 (20%)	20
Z-File			
Adult	84 (67%)	16 (13%)	100
Young	11 (9%)	14 (11%)	25
Total	95 (76%)	30 (24%)	125

to the number of early-onset female victims, not only for the R- and K-files, but also in the 193 individuals studied by Zigas and Gajdusek (though not in the Z-file, for the reason given in the previous paragraph). We shall return to this point when we come to consider the genetical hypothesis.

The remarkably good general agreement between these very differently obtained series of data may help to allay somewhat the very reasonable doubt as to the reliability of the Moke-Kagu (R-file) survey which was so largely dependent on native diagnosis and memory.

Table 5 shows the 2 x 2 classification according to sex and age of the 635 individuals recorded in the R-file as having died of a cause other than kuru. It may be seen that more than one-fifth of these deaths occurred in childhood. Of the 438 females recorded in the R-file as having died from kuru, tokabu or other given cause, 78 died when under 17 years of age and only 21 of these were kuru victims. Thus, about one-eighth of all females die from causes other than kuru before they reach 17 years of age. The chance of a woman dying from a cause other than kuru before the average age of late-onset of kuru must be greater than this and it may be much greater.

Adult Sibships

If kuru makes a greater relative contribution to the death rate in adult than in young females, then the proportion of female deaths due to kuru should be larger in adult than in young families. In order to test this, records of "adult-sibships" in which all living members had reached the age of 17 years were abstracted from the R-file. The numbers and classification of the members of these sibships are shown in Table 6. It may be seen that 13 out of 115 (i.e. 11 per cent) of the male deaths and 81 out of 142 (i.e. 57 per cent) of the female deaths in these sibships have been caused by kuru. This sex distribution is very similar to that already described for the entire R-file. However, relatively more of the female victims are recorded as having died as adults than is the case for the whole data. This difference has possibly arisen in part from our practice of recording deaths of ancestral relatives as having occurred in adulthood rather than in childhood whenever there was any uncertainty.

It is to be noted that 102 out of 227 (i.e. 45 per cent) of the males but only 46 out of 198 (i.e. 23 per cent) of the females were still alive at the time of study. In other words there were twice as many male as female members of these sibships alive and unaffected by kuru.

Family Size and Sex-Ratio

Table 7 summarizes the position in respect of family size and sex ratio in the offspring of the various groups of mothers in the three files.

The largest mean family size and the most extreme sex ratio both occur in the families of female kuru victims recorded in the Z-file. There is, in fact, a highly significant difference between the sex ratios in the offspring of these women and in those of all other women in the Z-file ($\chi_1^2 = 6.5$, $P = 0.01$). An examination of the Z-file shows that ascertainment of the families recorded in this file was very biased. Ten of the 62 families were ascertained through females said to have recovered from

TABLE 5. DEATHS RECORDED AS NOT DUE TO KURU IN THE R-FILE

	Females	Males	Total
Adult	205 (32%)	295 (47%)	500
Young	57 (9%)	78 (12%)	135
Total	262 (41%)	373 (59%)	635

TABLE 6. CLASSIFICATION OF MEMBERS OF ADULT SIBSHIPS (R-FILE)

Sex	Age	Alive	Dead				Totals
			Kuru	Tokabu	Other cause	Cause unknown	
F	Adult	46	75	7	38	10	176
	Young	—	6	1	15	—	22
	Total	46	81	8	53	10	198
M	Adult	102	5	18	73	10	208
	Young	—	8	1	10	—	19
	Total	102	13	19	83	10	227

TABLE 7. FAMILY SIZE AND SEX RATIO IN THE OFFSPRING OF VARIOUS GROUPS OF MOTHERS

Source	Cause of Death of Mothers if Deceased	Number of Mothers	Total Number of		Average Family Size m ± 2 s.e. (m)	Sex Ratio Female:Male
			Daughters	Sons		
R-file	Kuru	113	149	173	2.85 ± 0.30	100:116
	Tokabu	15	20	30	3.33 ± 0.78	100:150
	Other causes	126	159	220	3.01 ± 0.30	100:138
	Unknown	22	28	35	2.86 ± 0.82	100:125
	Alive	189	274	289	2.98 ± 0.28	100:105
K-file	Kuru	7	6	8	2.00 ± 0.62	—
	Other causes	2	2	3	—	—
	Unknown	3	4	2	—	—
	Alive	10	18	15	3.30 ± 1.12	—
Z-file	Kuru	60	73	129	3.37 ± 0.50	100:177
	Other causes	22	29	39	3.09 ± 0.82	100:134
	Unknown	15	19	17	2.40 ± 0.84	100:89
	Alive	52	78	90	3.23 ± 0.46	100:115

kuru, whereas there was only one such person included in the much larger R-file. Of the other 52 families, no less than 17 (i.e. 33 per cent) were ascertained through male victims of kuru. In 10 of these 17 families, the mother of the propositus also had died of kuru and these 10 women altogether had produced three daughters and 30 sons. In these 17 families there were 15 other married women who had died of kuru and they had 18 daughters and 24 sons. Including one woman said to have recovered, there were five others in the Z-file who were victims of kuru and who had a son who had died of it. These five women altogether had four daughters and 10 sons. The other 52 mothers in the Z-file who have died of kuru, but who have not had a son die of it, have produced 72 daughters and 97 sons. The question thus arises as to whether the unusual sex ratio in the offspring of the 15 females who have had a son die of kuru and have themselves died of it, is intrinsically important or is the result of selection of such families for inclusion in the Z-file. We believe that the latter is the case. Gajdusek in a personal communication has indicated that because of his pediatric interests it was often through young boys that he was led to the families recorded in the Z-file. And we have seen that it is in those families ascertained through male victims of kuru that the greatest excess of sons is found.

No criticism is intended by these comments on Zigas and Gajdusek's work. The very valuable pedigrees, which they have, most generously, made available to us, were not compiled with the intention of subjecting them to an analysis such as has been described here. The object was simply to obtain and record as many current cases of kuru as possible. This aim was achieved most successfully. However, in the process, some unusually striking pedigrees were collected with a frequency out of all proportion to their occurrence in the population. The procedure adopted for constructing the R-file was designed so as to avoid any such bias.

Deaths of Offspring of Males Dying of Kuru

Because of the early onset of kuru in males, affected individuals seldom become fathers. Only four such cases were included in the R-file, and in three of these the wife also died of kuru. These three familes consisted of i) a single son who died when aged one, ii) two daughters each of whom died at about one year, and iii) a single son aged six and still alive at the time of study. The fourth family, where the mother and children were still alive at the time of record, consisted of a boy aged seven and a girl aged five. The only family of this kind included in the Z-file was the same one as that described under ii) above. No such family was included in the K-file.

Deaths of Offspring of Women Dying of Kuru

The average age at death of early-onset female cases is even lower than that of male victims. However, the fact that females often marry when very young makes it possible for some early-onset victims to become mothers before succumbing to the disease. The data of the R-, K- and Z-files provide no information on how often this may happen, since they do not include estimates of age at death for deceased mothers. All mothers who died from kuru have been included in the late-onset class. However, it seems likely that very few early-onset victims were misclassified as late-onset because of this. Zigas and Gajdusek's data on age distribution in 193 female victims

included only seven individuals said to have been between the ages of 13 and 17 years inclusive.

The position in respect of deaths of offspring of women who have or have not died from kuru (and whose husbands have not died from it) is summarized in Tables 8 and 9. A number of striking, and we believe, very significant points emerge. Firstly, as far as the R-file is concerned, a comparison of Parts A and C of Table 8 reveals that kuru caused the deaths of 12 of the 66 sons of women dying of kuru as against only one of the 114 sons in Part C. Kuru also killed two-thirds (18/27) of the adult daughters of women dying of kuru as compared with less than half (45/95) of the adult daughters in Part C. It may also be noted that six of the 24 female deaths from

TABLE 8. DEATHS OF OFFSPRING OF WOMEN WHOSE HUSBANDS HAVE NOT DIED FROM KURU (R-FILE)

A. Where the women have died from kuru

Age of Offspring at Death	Daughters			Sons		
	Kuru	Tokabu	Other cause	Kuru	Tokabu	Other cause
Adult	18	2	7	3	4	19
Young	6	—	19	9	1	30
Total	24	2	26	12	5	49

B. Where the women have died from tokabu

Adult	5	1	2	1	2	5
Young	2	—	3	3	—	4
Total	7	1	5	4	2	9

C. Where the women have died from causes other than kuru or tokabu

Adult	45	11	39	—	13	73
Young	3	2	9	1	3	24
Total	48	13	48	1	16	97

TABLE 9. DEATHS OF OFFSPRING OF WOMEN WHOSE HUSBANDS HAVE NOT DIED FROM KURU (Z- AND K-FILES)

A. Where the women have died from kuru

Age of Offspring at Death	Daughters			Sons		
	Kuru	Tokabu	Other cause	Kuru	Tokabu	Other cause
Adult	8	—	—	11 + *1*	1	12 + *1*
Young	6	—	5 + *1*	9 + *1*	—	14
Total	14	—	5 + *1*	20 + *2*	1	26 + *1*

B. Where the women have died of causes other than kuru or tokabu

Adult	15 + *1*	—	5	—	3	4
Young	1	—	2	1	—	4
Total	16 + *1*	—	7	1	3	8

Note: The numbers in italics refer to the K-file. All other numbers refer to the Z-file.

kuru in Part A occurred in young girls, this being four times the incidence in Part C, (3/48).

The offspring of the small group of women dying of tokabu (Table 8 B) present a most interesting picture since, although the numbers are very small, the situation does seem to resemble that in Part A of the table rather than Part C. Of the 15 sons recorded, four died from kuru. Two of these individuals were brothers and one of their two sisters also died from kuru. A third male victim included in Table 8 B was his mother's only child. Tokabu, as mentioned earlier, is a form of ritual murder performed on an individual believed to have induced kuru by sorcery. The resemblance found here between Parts A and B of Table 8 may well be coincidental, but it deserves further investigation.

A peculiar feature of Table 8 A is the high proportion of deaths from causes other than kuru or tokabu said to have occurred at a young age. Mothers who died from causes other than kuru or tokabu had 48 daughters who likewise died from other causes, and only nine (i.e. 19 per cent) of these died before 17 years of age. However, where the mothers have died from kuru, as many as 19 (i.e. 73 per cent) of their 26 daughters who died from causes other than kuru or tokabu did so before 17 years. There is a similar difference for sons. Examination of the birth order and age at death of these children suggests that, irrespective of the cause of the mother's death, about one half died following the loss of their mother. Fifteen of the 19 deaths in female children, and 18 of the 30 deaths in male children, occurred in those less than five years of age. At least five of these 15 females and 10 of the 18 males died some time before their mothers. These findings, together with the fact that kuru has not been described in children under four years of age, leads us to raise the question as to whether, in fact, kuru can occur in very young children without the usual symptoms being observed.

The same essential features found in Table 8 are also present in Table 9. Thus, in the K- and Z-files, kuru caused the death of 22 of the 50 sons of women dying of kuru as compared with only one of the 12 sons of women dying from other causes. Also, six of the 14 deaths from kuru among the daughters included in Table 9 A occurred in young girls as compared with only one out of 17 in Table 9 B.

A GENETIC HYPOTHESIS

Analysis of these data not only suggested that a genetic basis was possible but also that a simple model could tentatively be proposed.

Our suggestion is that kuru is under the control of a single autosomal gene K dominant to its allelomorph *k* in females and recessive in males. In other words, males of the genotype KK are potential victims of kuru whilst those who are Kk or kk are clinically normal. Females of the genotype KK are potential early-onset victims, those of the genotype Kk are potential late-onset victims, and those who are kk are normal.

When the 250 pedigrees included in the R-, K- and Z-files were examined in the light of this hypothesis, no situation was found which was incompatible with it. An example of an extensive Fore pedigree is shown in Fig. 5. Clearly, pedigrees which

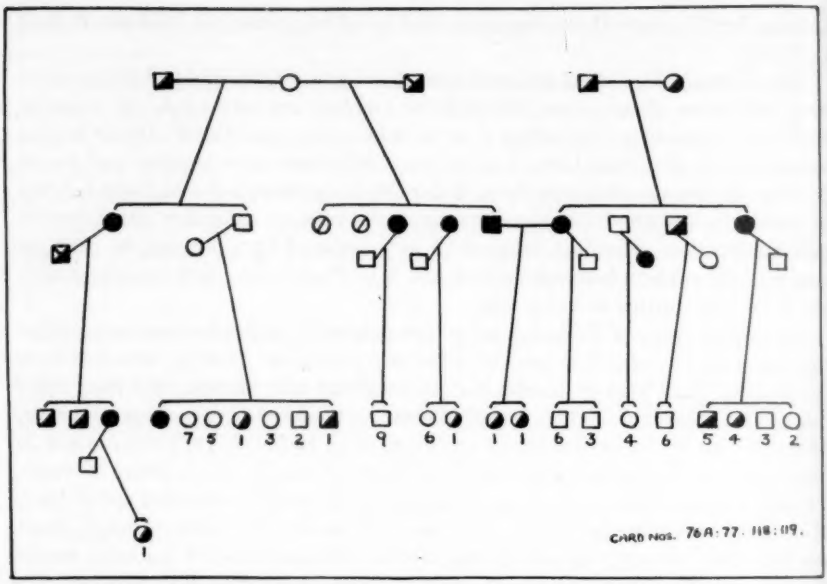


FIG. 5. Pedigree of a Fore family extending over four generations. Symbols: ● died from kuru; ◒ died from tokabu; ◕ died from causes other than kuru or tokabu; ⊘ cause of death unknown; ○ alive.

provide examples of the junction of kuru and kuru-free lines would be of particular value for testing the genetic hypothesis. However, it is extremely difficult to find such pedigrees. The reason for this is that within the Fore area, kuru is extremely widespread, and in the border areas where some intermarriage with non-Fore occurs, this has been the custom for some considerable time. Hence, we cannot be certain that such non-Fore people are not carrying the gene K in low frequency. However, there is one pedigree included in the R-file (Fig. 6) which seems to provide a possible example of the junction of kuru and kuru-free lines. This records the marriage of a Fore man with an Iababi woman who was born and had lived, until her marriage, some distance away from the Fore region. Presumably the man was a heterozygote Kk. A follow-up study of this family could be of very great interest indeed.

The birth of homozygotes KK to parents who are heterozygotes Kk would account for the phenomenon of "anticipation" or the occurrence of the disease at an earlier age in the second generation, which has been observed and remarked upon a number of times by Gajdusek and Zigas (1957, 1958). Also, homozygotes KK should be produced with the same relative frequencies in both sexes. We would therefore expect the number of male victims of kuru to be approximately equal, or (taking account of the excess of male over female births) slightly greater than the numbers of early-onset female cases. This is indeed so for the R- and K-files, and in the series of 193 kuru victims observed by Zigas and Gajdusek. In the Z-file, however, there are 30 male but only 11 early-onset female victims. This large difference might well be due

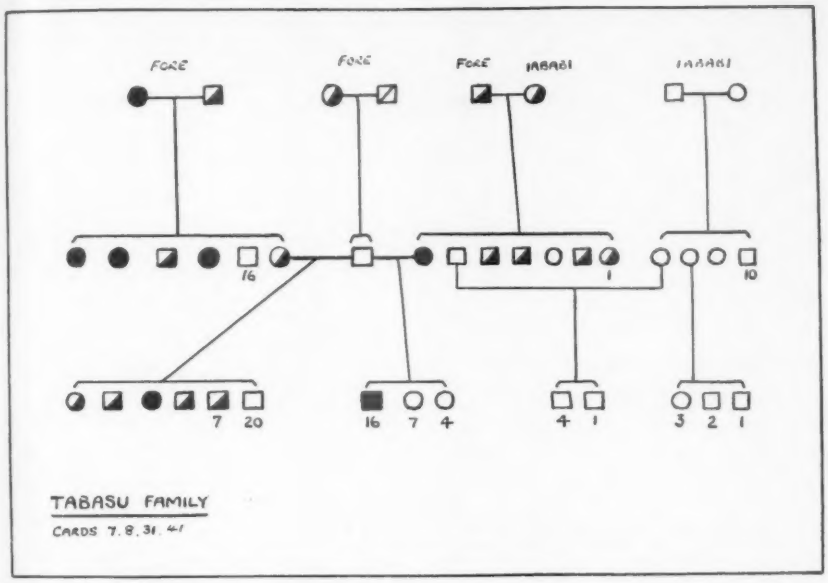


FIG. 6. Pedigree of the Tabusu family, a possible example of the junction of kuru and kuru-free lines.

to the biased ascertainment of the pedigrees in the Z-file which we have already described.

If kuru is, in fact, genetically determined in the way we have suggested, then any male with kuru, being a homozygote KK, must have had a mother whose genotype was either KK or Kk and in either event she would have been a potential victim of kuru. Some of these mothers would die of other causes before kuru could be manifested, but we cannot say what proportion of these potential kuru victims would be lost in this way.

Table 10 A gives the recorded causes of death of mothers of male victims of kuru in the R-, K- and Z-files. It may be seen that the situation is quite compatible with the genetic hypothesis. Five of the 28 mothers died of causes other than kuru (and it is of some interest that three of these five maternal deaths were due to tokabu).

As to sisters of male victims of kuru, we would expect, on the basis of our hypothesis, that one-quarter would be potential early-onset victims (KK), one-half potential late-onset victims (Kk), and the other quarter normal (kk). Our data included records of death of 15 sisters of male victims of kuru. Ten of these females died from kuru, three when very young and seven as married women. This is in very good agreement with the expectation.

Table 10 B shows the deaths of mothers (KK or Kk) of young females who died from kuru. It may be that not all of these young females have the genotype KK since there is presumably some overlap between the early- and late-onset classes. Any

individual included in this class on the basis of the age of onset of kuru and who was actually an heterozygote Kk could, of course, have derived the K gene from the father and so the mother need not have been a potential kuru victim. This factor, added to the general risk of death from other causes already mentioned, would lead

TABLE 10. CAUSES OF DEATH OF MOTHERS OF KURU VICTIMS

A. Mothers of male victims

	Kuru	Tokabu	Other Cause
R-file	9	3	1
K-file	2	—	—
Z-file	12	—	1
Total	23	3	2

B. Mothers of "early-onset" female victims

R-file	6	3	2
K-file	—	—	—
Z-file	4*	—	1
Total	10	3	3

C. Mothers of "late-onset" female victims

R-file	16	5	34
K-file	—	—	1
Z-file	8	—	11
	24	5	46

* Plus one "recovered kuru."

TABLE 11. CAUSES OF DEATH OF GRANDMOTHERS OF KURU VICTIMS

A. Grandmothers of male victims

	Maternal G. M.			Paternal G. M.		
	Kuru	Tokabu	Other cause	Kuru	Tokabu	Other cause
R-file	2	—	4	—	—	5
K-file	—	—	—	—	—	—
Z-file	3	—	1	2	—	1
Total	5	—	5	2	—	6

B. Grandmothers of "early-onset" female victims

R-file	2	—	6	—	—	6
K-file	—	—	—	—	—	1
Z-file	4	—	—	—	—	2
Total	6	—	6	—	—	9

C. Grandmothers of "late-onset" female victims

R-file	2	—	6	—	—	12
K-file	—	—	—	—	—	—
Z-file	2	—	1	—	—	2
Total	4	—	7	—	—	14

one to expect a somewhat lower incidence of actual deaths from kuru among the mothers of this class (Table 10 B) as compared with the mothers in Table 10 A. It may be seen that the observations (10 out of 16 mothers dying of kuru) are in tolerably good agreement with the hypothesis.

Table 10 C records the deaths of the mothers of adult female victims of kuru. The latter, presumed to be of the genotype Kk, could have derived the K gene from either the mother or the father. The actual proportion of mothers of this group which would be expected to be heterozygotes Kk cannot readily be determined since it depends on the population frequency of the gene K and possibly on selective forces which may be operating. And, as before, death from other causes prior to the onset of kuru would tend to reduce the proportion of deaths from kuru below the expected value. The actual observation is that 24 out of 75 mothers in this group died from kuru.

Table 11 records the deaths of grandmothers of kuru victims in the three files. As in the last case, the determination of the expected frequency of heterozygotes Kk among grandmothers is a problem in population genetics and one which we shall not pursue here.

DISCUSSION

The genetic hypothesis which we have advanced for kuru provides a ready explanation for the family data which have been collected so far. However, our study must not be regarded as anything other than preliminary. More family studies are certainly called for.

On the basis of the genetic hypothesis, predictions can be made as to the likelihood of kuru developing in a considerable number of individuals who are now alive and are recorded in our pedigree files. Follow-up studies of these individuals are clearly indicated. If males and young females with kuru are, in fact, homozygotes KK, careful study of both parents would be of extreme importance in the search for some feature which might identify the presence of the K gene, other than by the development of the disease. Study of all available pathological material should be made to determine whether there are any recognizable differences between male and young female cases on the one hand, and adult female on the other. Careful study of the few cases of alleged recovery from kuru should be made to determine whether or not these are heterozygotes Kk in which the gene does not have complete expression. The high death rate in childhood among the offspring of women dying of kuru (Table 8) requires explanation. The speculation arises as to whether some as yet unrecognized form of kuru, or some condition related to it, is occurring amongst these children. Also, the precise relationship between deaths from kuru and those from tokabu needs to be explored.

Should our hypothesis prove to be essentially correct, this will be one of the most curious genetic situations found in a human population. In this case, study of the population genetics could be expected to throw light on the evolutionary aspects of the problem. Since kuru occurs with a very high frequency among the Fore, it may be that powerful selective forces have been, and possibly still are, associated with it, but the genetic basis would need to be definitely established before these and other

aspects of the population problem could be examined properly. However, it is of interest to note that for an idealized population in which there were random mating and no selective differences and where the gene K had a frequency of 37 per cent the genotypes KK, Kk, and kk would have frequencies of 14, 47 and 39 per cent respectively, values not unlike those suggested for the data of the R-file. Clearly, however, the deaths of males and young females who are homozygotes KK would be removing a not inconsiderable number of K genes from the population in each generation.

The control of kuru in New Guinea raises difficult administrative as well as medical problems. If our hypothesis is correct, intermarriage of the Fore with members of kuru-free groups in other parts of the island would lead to a reduction in the total number of kuru victims seen during the first twenty years or so, when fewer homozygotes KK would be produced, but this would be at the expense of spreading the gene K to these other groups. It would thus be as well to keep a very close watch indeed on any movement of the Fore outside their present region.

For a disease with the features and frequency of kuru, the satisfactory demonstration of a genetic basis would be a matter of some difficulty under any circumstances. Among the Fore, it would be even more difficult. Our genetic hypothesis is therefore advanced but tentatively at this stage. The main justification for putting it forward at all now is the hope that it may assist in the direction of further investigations into this unique disease which is a tragic problem for the Fore people and a challenge to modern medicine.

SUMMARY

Kuru is a rapidly progressive neurological disease which has been found only amongst the Fore natives of New Guinea where it has a very high frequency. It seems that kuru is at present responsible for the deaths of about one half of the Fore females and about one tenth of the males in that part of the region where population frequencies have been obtained. From the time of onset of the symptoms of kuru death usually follows within a few months. Kuru has not yet been observed in any child under four years of age but it has been found in natives of all other ages. In males the distribution of the age of onset seems to be unimodal with the mean age at about 14 years. In females many more cases have occurred in adults than in children. Here the age distribution is bimodal one mode occurring at about eight and the other at about 33 years. A large body of genealogical data bearing on kuru has now been collected and their analysis and interpretation are described in this article. On the basis of the analysis it is suggested that kuru may be under the control of a single autosomal gene *K* dominant to its allelomorph *k* in females and recessive in males. In other words males of the genotype KK are potential victims of kuru whilst those who are Kk or kk are clinically normal. Females of the genotype KK are potential "early-onset" victims, those of the genotype Kk are potential "late-onset" victims and those who are kk are normal. This simple genetic model for kuru has some very interesting implications for further research into this tragic disease.

ACKNOWLEDGMENTS

The field investigations on which this work is based were made possible by the generous assistance of the University of Adelaide and the Australian Department of

Territories. We are indebted to many members of the Administration of Papua and New Guinea, particularly Dr. R. F. R. Scragg, Director of Health, and Mr. J. Baker, Patrol Officer, Okapa, for invaluable assistance. Grateful acknowledgement is due to Drs. V. Zigas and D. C. Gajdusek who freely made their unique information and experience available to us.

REFERENCES

BENNETT, J. H., RHODES, F. A. AND ROBSON, H. N. (1958) Observations on kuru, I. A possible genetical basis, *Australas. Ann. Med.* 7: 269.

BERNDT, R. M. (1954) Kamano, Iate, Usurufa and Fore kinship of the eastern highlands of New Guinea: A preliminary account, *Oceania*, 25: 23.

BERNDT, R. M. (1958) A devastating disease syndrome: kuru sorcery in the eastern highlands of New Guinea, *Sociologus*. 8: 4.

FOWLER, M. AND ROBERTSON, E. G. (1959) Observations on kuru, III. Pathological features in five cases. *Australas. Ann. Med.* 8: 16.

GAJDUSEK, D. C. AND ZIGAS, V. (1957) Degenerative disease of the central nervous system in New Guinea, *N. England J. M.* 257: 974.

GAJDUSEK, D. C. AND ZIGAS, V. (1958) Untersuchungen über die Pathogenese von Kuru, *Klin. Wschr.*, 36: 445.

GREENFIELD, J. G. (1954) *The Spino-cerebellar Degenerations*. Blackwell, Oxford.

SIMPSON, D. S., LANDER, H. AND ROBSON, H. N. (1959) Observations on kuru, II. Clinical features, *Australas. Ann. Med.* 8: 8.

ZIGAS, V. AND GAJDUSEK, D. C. (1957) Kuru: Clinical study of a new syndrome resembling paralysis agitans in natives of the eastern highlands of Australian New Guinea, *Med. J. Australia* ii: 745.

ZIGAS, V. AND GAJDUSEK, D. C. (1958) Personal communication.

Idiopathic Dystonia Musculorum Deformans

I. The Hereditary Pattern

W. ZEMAN, R. KAELBLING, AND B. PASAMANICK

WITH THE COLLABORATION OF

J. T. JENKINS

Research Division, Columbus Psychiatric Institute and Hospital, Department of Psychiatry, College of Medicine, The Ohio State University

INTRODUCTION

IT IS GENERALLY AGREED that dystonia musculorum deformans may be associated with a number of well delineated pathological entities, such as Wilson's disease, various of the encephalitides, vascular malformations, idiopathic calcification of cerebral vessels, and others (Hallervorden, 1957). In contradistinction to those "symptomatic" forms of dystonia this paper is concerned with the idiopathic form to which the term dystonia musculorum deformans should be confined according to Herz (1944).

Dystonia is conventionally classified as an extrapyramidal disease. The ratio between affected males and females is about 60:40. No explanation for this sex distribution has been offered, as yet. The disease usually begins during childhood or adolescence.

The chief symptoms are dystonic posture and dystonic movements. The latter are true hyperkinesias and are characterized by relatively slow, long sustained, powerful, nonpatterned, contorting activities of the axial and appendicular muscles. The muscles most commonly involved are those of the neck, trunk, and proximal portions of extremities. Involvement of unilateral muscle groups often results in bizarre torsion movements, hence the alternative term "torsiondystonia" for the disease. Volitional acts are often seriously compromised. The dystonic movements disappear during sleep and under general anesthesia. "Dystonic posture" is the term used if the end position of a dystonic movement is maintained for any length of time. Eventually this may lead to contracture deformities. Various dystonic postures are exemplified by Fig. 1.

The onset is gradual, with drawing sensations in axial and appendicular muscles. Gait is usually impaired first, but writer's cramp or torticollis may also constitute the initial symptom. Muscle hypertrophy may result from continuous activity, eventually leading to such deformities as kyphoscoliosis, pes equinovarus and others. Speech is often impaired. Sensation and mentation are rarely affected. Laboratory findings are within normal limits. The course varies, ranging from complete incapacitation to a mild nonprogressive illness. Sometimes remissions may occur.

The earlier reports on the disease noted Jews to be exclusively affected. Subsequent studies, however, invalidated this notion.

Received September 26, 1958.

FIG. 1. Family P (see Fig. 2) Standing from left to right: IV-10. Note the dystonic posture of head, trunk and legs which has resulted in a moderate kyphoscoliosis and inversion of the feet V-10. Appears normal on this picture. V-14. Appears normal on this picture. V-11. Note spastic torticollis and dystonic posture of left arm. Sitting, V-13. Severe kyphoscoliosis and torticollis. Dystonic posture of left hand. Acquired bilateral pes equino-varus.

Schwalbe (1908) who reported the disease in three siblings is usually credited with the discovery of the condition, although Gowers had described the disease in two brothers as early as 1893. Despite this familial occurrence, Mendel (1919) in the first monograph on the subject mentioned that the condition was obviously not inherited. This somewhat surprising conclusion probably stemmed from the authoritative statement of Oppenheim (1911) who reported four instances of the disease, all of which were sporadic. He also mentioned the cases of two siblings who exhibited a clinical picture somewhat similar to the other cases. However, Oppenheim was not certain as to the diagnosis in those cases. In his textbook, Oppenheim (1923) stated that nothing definite was known regarding the heredity of the condition, although siblings had been affected in two instances. A similar view was expressed by Herz (1944) in his classic study of the disease.

On the other hand, Polish and Russian authors consistently proposed dystonia as a definitely inherited disease. Mankowsky and Czerny (1929) reported on two families, the first with a seemingly recessive, the second with a dominant pattern of inheritance. Regensburg (1930) described four families, among them the original observation of Schwalbe (1908), in which the disease occurred in the form of a Mendelian dominant.

Beilin (1934) examined 41 members of a family of 104 in which the disease was recessively inherited.

The purpose of this communication is to present evidence that idiopathic dystonia is definitely inherited and is transmitted predominantly as a Mendelian dominant. The second contention is suggested by the inheritance pattern of a family reported here for the first time, and is in line with most of the previously known instances of familial occurrence of the disease.

FAMILY HISTORY

The family history was obtained as fully as possible, although a certain lack of cooperation was encountered among some of the members. The chief problem was a reluctance to admit to a familial disabling disorder.

The family is of English extraction and, as far as could be ascertained, has lived in rural Ohio for at least six generations. Consanguinity was not found during this period of time. An effort was made to examine each descendant of the first person known to have been affected by the disease (III-16 in Fig. 2). Furthermore, the siblings of those individuals and their descendants who came from unaffected families, but had produced diseased children (III-3 through 9, IV-1 through 9 and VI through 8 in Fig. 2), were questioned and examined whenever possible.

It is felt that the information set forth in the family tree (Fig. 2) is generally accurate. In those instances in which the individual could not be examined the information was corroborated by at least two independent informants. This is invariably true for that part of the family in which the disease is inherited in a direct line.

EXPLANATION OF THE PEDIGREE

General Information. A cross indicates that the individual was deceased at the time of the investigation. The age indicates either the age at the time of death or the age in 1958, the time of investigation. The heavy lines indicate the connections between the various generations through which the disease has been passed down.

Specific Information

I 1 died of old age; 2, 3, and 4 no information available.

II 1 died of old age; 2 no information available; 3 and 4 died of old age.

III 1 died of old age; 2 died of heart failure; 3 through 7 and 9 were healthy individuals. Information regarding this family was obtained from the children of 5 and 7 who stated that there is no nervous or mental disorder known in the offspring of these six persons, who number more than 100 at the present time. Eight has been described by members of his family as a healthy, normal individual; by members of his wife's family as a periodic drunkard who would spend all his money on liquor and then would work for a number of weeks. He was a very poor provider. All his children were either brought up in foster homes or in the County Children's Home.

Ten died as an infant; 11 died at age 9 from an accident. Twelve through 15 and 17 through 20 were eight siblings having descendants numbering more than 100, none of whom is or has been affected by dystonia or by any other

FIG. 2. Family P. See text for explanation.

nervous or mental disease. This information was obtained from 18 and from the widow of 20 and was corroborated by several of their descendants. Sixteen is the first known case of dystonia in this family. The patient showed the first signs of the disease during childhood, allegedly about age 10, and was totally disabled at 23. She died at 30, reportedly from pneumonia.

IV 1 through 6 and 8 have had 10 children, 19 grandchildren and one great-grandchild. All these individuals are normal. There are no signs of nervous disease in this family. This information was obtained from 4 and 5. Nine died as an infant.

Ten has been mildly afflicted with dystonia since age 21 (Fig. 1). The condition has been stationary since. Eleven has never been sick and was killed when his airplane crashed. Twelve had severe dystonia from age 5 on and died, completely crippled, in the County Children's Home at age 20. This child is described as having been similarly affected by the disease as was his mother, III-16. Thirteen showed the first signs of the disease after the delivery of her third child at age 25. Dystonic symptoms have since been stationary after a progressive course of approximately one year. Fourteen could not be examined. According to her own statement, corroborated by an aunt, she has never shown any signs of the disease. Fifteen died at age 3 weeks.

V 1 through 8 are healthy and are not afflicted by nervous or mental disorders, according to statements by 5 and 6 who have been examined.

Nine has a clubfoot, surgically corrected, but no other defects. Dystonia developed in 10 (Fig. 1) after the birth of her seventh child at age 31 and has been practically stationary since. Eleven (Fig. 1) showed first signs of dystonia at age 17 at which time St. Vitus dance was diagnosed. He is able to work, however. Twelve has not been examined and is allegedly healthy. Severe dystonia began in 13 (Fig. 1) at age 11 and she is now completely crippled. Fourteen (Fig. 1) has mild dystonia in the form of temporary limping, blepharospasm, and mild torticollis. Fifteen and 16 have not been examined; they are allegedly healthy. Seventeen developed a temporary limp at age 10, which has now subsided. This person is in poor general health at present. She has not been examined, but probably has dystonia. Classic dystonia began in 18 at age 9, and since age 13 he has been completely crippled. Severe dystonia began at age 5 in 19, who is now completely crippled. Twenty and 21, identical twins, are both healthy. Twenty-two through 27 have not been examined and are allegedly healthy. Twenty-eight has not been examined. According to information obtained from the attending psychiatrist, this boy is severely neurotic and retarded. There are, however, no signs of dystonia.

VI 1 through 3 are bright and intelligent children. Four, 5, and 7 have speech difficulties, cannot pronounce "f" and "s" and are mentally retarded to the extent that they entered public school with a delay of two years. Six has overt dystonia, developed at age 5. He is not crippled, but has a typical dystonic gait. Eight through 10 are allegedly healthy. Eleven has severe mental retardation and is now attending the first grade. This child shows several stigmata in the form of abnormal position of teeth and pronounced prognathy,

but has no signs of dystonia. Twelve through 19 are allegedly healthy. Twenty has bilateral congenital metatarsi vari. Twenty-one through 27 are allegedly healthy.

ANALYSIS OF PEDIGREE

It is evident from the pedigree that there is marked evidence for the inheritance of dystonia musculorum deformans, the disease having been transmitted through at least four successive generations. It is very probable that some members of the last generation born to an affected parent and recorded here as free of the disease will ultimately develop it, for the experience of this family shows that the onset in some instances has been delayed to as late as the age of 31. On the other hand, it has never appeared earlier than the age of five.

Beginning with the first instance of the disease in III-16, three of her six children developed dystonia. Of the 14 children of IV-10, six or possibly seven have the disease. Apparently 50 per cent of the offspring of two successive generations are affected. This, of course, suggests a Mendelian dominant which, at least in these generations, shows complete penetrance. That the three children of IV-13 and her four grandchildren have been free of the disease does not refute such a mechanism of inheritance.

Within this family there is a considerable variability of expressivity of the disease. For instance, V-14 shows symptoms of dystonia in a very mild form, manifested by temporary limping, torticollis and blepharospasm. At times this patient appears almost normal. On the other hand, three of his siblings are totally crippled and helpless. V-10 has neither spontaneous movements nor the typical dystonic posture. Yet, upon performing certain volitional movements, typical dystonic features can be easily elicited. Certainly in cases like these two, one could take the position that such manifestations do not justify the diagnosis of dystonia. Yet, it would be illogical to consider any other diagnosis in view of the fact that grandmother, father, siblings, and one child are so definitely affected by dystonia. Applying "Occam's razor" of scientific parsimony, it is certainly the most logical conclusion that this family exhibits "formes frustes" as well as full-fledged cases of dystonia. Obviously, the strict diagnostic criteria as put forth by Herz (1944) were not applied to the subject cases. Whether the congenital abnormalities of the feet encountered in cases V-9 and VI-20 are related to dystonia is a matter of speculation. The question will be discussed in more detail in a subsequent paper dealing with the clinical features of dystonia as observed in this family.

In conclusion, it seems safe to assume that in this family the disease is propagated by a Mendelian dominant gene, with varying expressivity, but probably with complete penetrance. The fact that the first affected of this family was a sister of eight healthy siblings, all of whom have healthy offspring, is difficult to explain. Since this patient was the seventh child of eleven, there is little probability of illegitimacy, so that a spontaneous mutation may be considered as the probable explanation.

REVIEW OF LITERATURE

There are at least 29 instances of familial occurrence of dystonia reported in the literature. In twelve of these the disease occurred only in members of one generation

(Abrahamson 1920, Bernstein 1912, Gowers 1893, Jakob 1924, Kehrer 1936, Oppenheim 1911, Price 1921, Santangelo 1934, Spiller 1913, Svejcar 1924, Taylor 1920, van Bogaert 1946). Kehrer (1936) suggested that his cases (three sisters) were the result of an exogenous factor in the form of a maternal disease with bouts of exacerbation during the pregnancies. Herz (1944) considered the observations of Gowers (1893) and Price (1921) as not proven instances of the disease. Oppenheim (1911) also was not certain as to the diagnosis in his cases, and finally van Bogaert (1946) considered the two brothers he examined to be instances of choreoathetosis with torsion spasms. Hallervorden (1957), however, included the latter cases in his discussion of dystonia.

This leaves seventeen families in which dystonia was observed in members of different generations. Again, Herz (1946) excluded a number of these, either on the basis of insufficient data or because he felt they belonged to other well defined morbid entities. The former exclusion applies to the family reported by Dawidenkow and Solotowa (1921) in which the father, his sister and one daughter suffered from what the authors considered was dystonia. In the case of Fossey (1922), to be discussed in detail later, Herz (1944) felt that there was sufficient evidence to suspect Huntington's chorea. The cases of Benedek and Rakowitz (1940) were complicated by congenital deformities of the face and might well constitute a different clinical syndrome. The clinical data in the cases of Munch-Petersen (1930) leave some doubt as to a diagnosis of dystonia.

The remaining thirteen families will be discussed in detail. Nine of them conform to the stipulations for a dominant inheritance pattern, (Beder 1926, Dzerschinsky 1925, Jankowska 1934, v. Keyserlingk 1956, Mankowsky and Czerny 1929, Regensburg 1930 (three families), van Bogaert 1940-41). Four are suggestive of a recessive mode of inheritance (Beilin 1934, Mankowsky and Czerny 1929, Santangelo 1934, Wechsler and Brock 1922).

DOMINANT CASES

By far the most convincing example of a dominant pattern of inheritance is the Lewin family (Fig. 3) independently described by Schwalbe (1908), Regensburg (1930), Jankowska (1934), Rose (1937), and Vogt (1942). This pedigree also exemplifies the pitfalls of research.

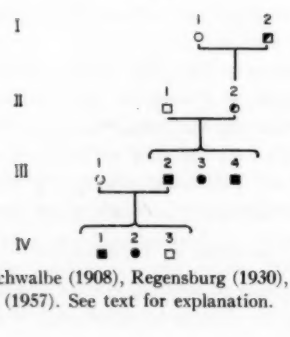


FIG. 3. Family Lewin after Schwalbe (1908), Regensburg (1930), Jankowska (1934), Rose (1937), Vogt (1942), and Hallervorden (1957). See text for explanation.

No information is available on I-1. I-2 suffered from tremor of the hands after having been buried alive in the Turco-Russian War. His son-in-law, II-1, was allegedly healthy but his daughter, II-2, also had shaking of hands and feet during her later years of life. III-2, 3, and 4 are the three siblings first described by Schwalbe (1908). III-3 died after an illness of sixteen years duration. Her brain was examined by C. and O. Vogt (1937), who found an atrophy of the small striatum cells, of the nucleus centralis thalami and of the astrocytes throughout the brain, with overgrowth of oligodendrocytes. III-4 committed suicide after six years of illness. III-2 was the least affected of the siblings. He showed his first symptoms at age 15, but his condition remained stationary thereafter. At age 33 he married a healthy girl, III-1, from a healthy family who bore him three children. The first son, IV-1, developed dystonia at age 6 and died 15 months later. His brain was examined by Rose (1937) who found lessions similar to but less pronounced than those described by the Vogts (1942). IV-2 became ill at age 2 and was still alive at age 9. IV-3 was healthy at age 7.

This family is a lucid example of how a critical and detailed genealogic study can reverse previous interpretations. Regensburg (1930) had already pointed out that Schwalbe (1908) had not paid any attention to the familial trend of shaking of the extremities, presumably because he could not understand Russian, which was the native tongue of these patients. Partly on the basis of this omission the hereditary character of the disease was seriously questioned by its most diligent students. Another important factor brought to light by this pedigree is the variable expressivity. III-2 was only mildly affected by the condition. The disease remained stationary for decades. This patient never developed speech disturbances. The dystonic cramps were painless. His two affected children, as well as his two siblings, ran an entirely different course and exhibited far more severe symptoms. In both children the disease was rapidly progressive, leading to an early crippling. Both suffered from painful spastic contractures, and IV-1 lost his speech completely. In this respect there is much similarity between these cases and IV-10 and his children, V-13, 18, and 19 of our reported pedigree.

On the basis of the anatomical findings and the apparent dominant pattern of inheritance, Hallervorden (1957) is inclined to consider this form of dystonia as a variant of Huntington's chorea. In support of this assumption, he quotes the family described by Fossey (1922) (Fig. 4). Unfortunately, this paper is fragmentary and the author's contentions are poorly documented. Most of the pertinent statements are quotations of W. G. Spiller's opinions, who apparently did not care to publish his observations. There is, however, a possibility that Fossey's cases might include those which were described by Spiller (1913).

After having become familiar with Oppenheim's original work, Spiller felt compelled to change the diagnosis of three siblings, all inmates of the Philadelphia General Hospital, from Huntington's chorea to dystonia. He pointed out that the importance of his observation rested upon the familial occurrence and the fact that the patients were not Jewish. (It will be recalled that in those days dystonia was believed to occur almost exclusively in Jews and not to be an inherited disease.) The members of the family reported by Fossey, were also inmates of the same

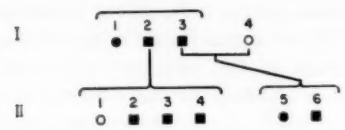


FIG. 4. Family of Fossey (1922). See text for explanation.

hospital. Furthermore, Spiller had diagnosed cases II-5 and 6 as instances of dystonia. The hospital records gave the diagnosis of Huntington's chorea for I-2 and 3, and II-2 and 3. I-1 was diagnosed as having suffered from nervous trouble.

The only point in favor of chorea in Fossey's case II-5 is the fact that the proband became sick at age 33. For the other family members, the age of onset is not given. While an age of onset of 33 does not rule out dystonia, it is nonetheless not the rule. Mankowsky and Czerny (1929) report on the father of two children with dystonia, who showed the first signs of the disease at age 32. On the other hand, when Hallervorden (1957) contends that the dystonia occurring in the Lewin family is a variant of Huntington's chorea, he ignores completely the fact that the latter disease begins between the ages of 21 and 60 in 94 per cent of all cases. Furthermore, hereditary chorea is extremely rare, if not nonexistent, among Jews.

The family on which Mankowsky and Czerny (1929) based their case for a dominant pattern of inheritance is shown in Fig. 5. II-2 became sick with choreatic and dystonic movements after he had lost his fortune at age 32. III-1 and 1 were twins. III-2 died as an infant and III-5 at age 7.

Here the solitary appearance of one diseased member in a sibship of six again suggests a spontaneous mutation, as stipulated for our III-16. III-1 and III-6 had classic dystonia. III-3 apparently had a *forme fruste*. These authors quote the observations of Beder (1926), a father and daughter with dystonia, and of Dzerschinsky, with father and son affected by the disease, as supportive evidence of dominant inheritance.

In addition to the famous Lewin family, Regensburg (1930) published three more instances of familial occurrence. The most striking is the family Frdk (Fig. 6). I-1 came from a healthy family. I-2 and 3 and II-3, 4, 5 all showed classic dystonia. III-1 and 2 in addition to dystonia exhibited signs of muscular dystrophia. II-1, 2, 6, 7, 8 had speech difficulties but otherwise no signs of the disease.

In the family Nsls, the father and two children were affected and in the family L. B., the father and four of his six children had dystonia.

Haenisch (1914) observed dystonia in father and son.

Van Bogaert (1940–41) reported on a mother and her two daughters who were affected by spastic torticollis. Several members of the family exhibited tremor and dystonic movements of varying degrees.

Von Keyserlingk (1956) described a father and four of his five children affected by dystonia. While two of the children were severely diseased, the father's symptoms were scant and practically restricted to speech difficulties. His sister was similarly affected; no information was available on seven other siblings. Von Keyserlingk

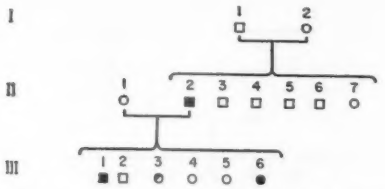


FIG. 5. Family II of Mankowsky and Czerny (1929). See text for explanation.

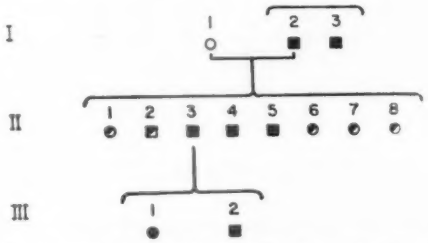


FIG. 6. Family Frdk of Regensburg (1930). See text for explanation

points out that the speech difficulties are *formes frustes* or abortive forms of the condition.

RECESSIVE CASES

The first observation was reported by Wechsler and Brock (1922). In their case #3 a remarkable familial tendency was discovered (Fig. 7). Unfortunately, the authors did not elaborate on the pedigree which they published. It was stated only that the grandparents in generation I were in good health. Because of the paucity of pertinent information on II-2 and 3 it is tempting to speculate on the possibility that these cases had a *forme fruste* of dystonia or that there was incomplete penetrance. In any event, the occurrence of the disease in the offspring of two supposedly unaffected siblings would require that each had married a heterozygote. However, the rarity of the disease, and the absence of consanguinity in the pedigree would suggest that the above explanations are preferable to that of the disease being a recessive in this family.

A somewhat similar situation exists in regard to the case of Mankowsky and Czerny (1929), on which they elaborated the hypothesis of a recessive inheritance (Fig. 8). I-2 was allegedly healthy. I-3 and 4 suffered from what was diagnosed as Huntington's chorea. II-2 died as an infant. II-3 and 4 were affected by dystonia. The same criticism we have made pertaining to the case of Wechsler and Brock applies to this observation.

The most diligently elaborated pedigree of a family with dystonia was published by Beilin (1934–35). It is too complex and large to be included here. Beilin found five cases of severe and four of mild dystonia among 104 members of one family

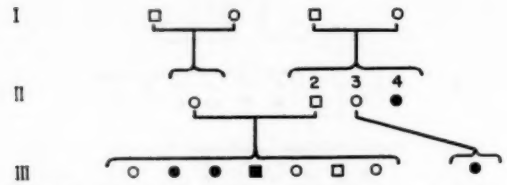


FIG. 7. Family of Wechsler and Brock (1922). See text for explanation.

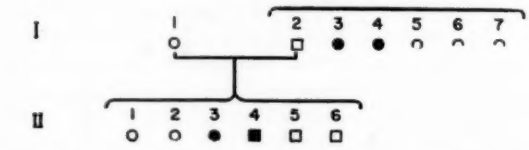


FIG. 8. Family I of Mankowsky and Czerny (1929). See text for explanation.

spanning six generations. The author applied rigid standards for the clinical diagnosis and did not speculate on the possibility of *formes frustes*. This is exemplified by his case D-10, the mother of two children with mild dystonia. This person suffered a contracture of one arm due to painful muscle cramps during adolescence which were never clarified etiologically. Yet she is listed as healthy and unaffected. There is one branch of this family in which consanguinity occurred. Unfortunately, all four children born to this couple died in infancy and childhood from epilepsy, but none showed any signs of dystonia. Another shortcoming of the chart of Beilin is the fact that some 30 members of the 5th and 6th generation of the family had emigrated to the United States, so that no information could be obtained first-hand. It is interesting to speculate on the possibility of some of these members having shown up in American medical journals as instances of dystonia. Finally, the author encountered considerable difficulties in obtaining information. The family members tried to dispel any suspicion of a familial disorder, pointing to impossible and ridiculous factors which were said to have precipitated the outbreak of the disease in the affected members. Therefore, it must be concluded that Beilin's family chart, although investigated with zeal and intelligence, is not necessarily proof of a recessive pattern of inheritance of dystonia.

The strongest case for such a contention was presented by Santangelo (1934). In this family three of five children born to apparently healthy parents were affected by the disease. The important fact is that the father's father and mother's father were first cousins. Nothing, however, is mentioned about ancestral and collateral generations.

It must be admitted that consanguinity increases the chances of both parents being carriers of a recessive gene, especially where a condition as rare as dystonia is concerned. Yet, considering the fact that marriages as close as second cousins are common in Italy, this case is only slightly indicative of recessive inheritance.

DISCUSSION

In evaluating the information from the literature and from the present report, one cannot escape the conclusion that there is much evidence in favor of a Mendelian dominant in the propagation of the disease. In fact, were it not for the observations of Santangelo (1934) and Beilin (1934–35), one would certainly be inclined to disregard whatever evidence had been presented in support of a Mendelian recessive. Even in the cases assumed to be recessive, but certainly in all those claimed to be dominant, the expected ratio of 50:50 of affected and nonaffected is met, with the notable exception of Beilin's family (1934–35).

As the situation stands now, it is probably safe to assume that in most instances a Mendelian dominant seems to be the mode of inheritance. But it is worthwhile to recall the statement of Kehrer (1936) that pure dominant and pure recessive inheritance are only the extremes of all possible modes of inheritance. Also, it should be remembered that several ailments are known in both dominant and recessive forms.

The study of the subject family and the review of the literature of the hereditary aspects of dystonia permit us to draw a number of other conclusions.

Clinical and Morbid Entity. The massed occurrence of dystonia in several families can be safely considered as evidence for a specific clinical entity. Whether dystonia is a morbid entity of course depends upon the pathomorphology of the condition. This could be investigated so far in only two instances of the Lewin family. The similarity of the histopathology in these two cases allows one to postulate a morbid entity at least for this family. Since Quandt (1956) and others were able to demonstrate identical findings in single, i.e. sporadic, cases of idiopathic dystonia, there is reason to assume that dystonia musculorum deformans actually represents a morbid entity.

On the other hand, it should not be ignored that van Bogaert (1946) examined two brothers with torsiondystonia and choreo-athetosis in which the lesions of the CNS were restricted to pallidum and corpus of Luys and, therefore, were entirely different from those described by the Vogts (1942), Rose (1937), and Quandt (1956). Furthermore, the various types and sites of pathologic lesions in cases of symptomatic dystonia provide ample evidence that the clinical syndrome can be produced by a number of differently located lesions and their combinations.

Thus, it appears safest to leave the question of the specificity of morbid entity unanswered at this time.

Further Classification. Herz (1944) proposed that dystonia could be further classified into an early form occurring shortly after birth, the juvenile form with the onset between 5 and 15, and the late form after 15 years of age. Experience with the hereditary cases of the disease shows that such classification is somewhat arbitrary, because in one and the same generation the disease might begin any time from birth to age 33 or even later. This situation which also prevails in other heredo-degenerations brings to mind the remark by van Bogaert (1948) that the heredo-familial diseases, whether congenital, precocious, or of late manifestation, are but the expression of one and the same genetic disorder. It depends on the degree and the moment

of intervention of one or a number of perturbing factors when the genotypical disturbances become phenotypically manifest. These perturbing factors are either other coexistent but autonomous predispositions (genotypical milieu, apparently dormant or "*restant*" genes), or are exogenous or "*peristatic*" causes.

Nonetheless, Herz's classification is not without merit, simply because it facilitates the prognosis of the further development of the condition. As demonstrated by the familial cases collected here, the disease usually runs a more severe course the earlier it becomes manifest. Conversely, the later the onset, the greater the chance for arrest or remission. This, however, is true only as a generality.

Clinical Forms of Dystonia. The marked variations in expressivity noted by practically all the investigators of affected families, calls for a revision of the hitherto accepted diagnostic criteria. Since the clinical manifestations of the afflicted members of the subject family will be treated in a separate communication, only a few pertinent points will be made here.

Herz (1944) gave the following criteria for the clinical diagnosis:

a) Selective systemic symptoms in the form of dystonic movements and postures.

b) Gradual development, without recognizable etiological factors at the onset.

Obviously, these rigid diagnostic criteria would not permit making a diagnosis in some cases in which there is evidence that the person had received as well as propagated the gene responsible for the disease. This is, for instance, true of our case V-10 who has no dystonic postures and exhibits dystonic movements only under certain restricted conditions of volitional movements. In other words, all *formes frustes* would be excluded if Herz's diagnostic criteria were accepted without reservation. However, it is exactly the *forme fruste* which is of the greatest importance, should attempts be made to eradicate the condition by eugenic counseling.

Secondly, the stipulation that the disease develops without recognizable etiologic factors could be misleading. Certainly, the etiology of the condition seems to be a heredo-degenerative process, but it must be kept in mind that the onset of the clinical manifestations can be "triggered" by any number of exogenous "*peristatic*" factors. This is illustrated by one of the patients of Mankowsky and Czerny (1929) and might also explain the cases of Kehrer (1936) and others.

It is, therefore, proposed to set more flexible standards as to the diagnostic criteria and to incorporate an evaluation of possible genetic as well as "*peristatic*" factors into the differential diagnostic considerations.

Sporadic Cases. It should not be ignored that the number of sporadic cases of dystonia by far exceeds the familial cases. This fact has certainly played an important role in obscuring the hereditary nature of the disease and seems, at least superficially, to argue against a dominant inheritance. It will be noted that in Huntington's chorea, the prime example of a dominantly inherited neurologic disease, the ratio is reversed. This apparent discrepancy can be resolved by the following arguments:

In the first place, it is important to remember that dystonia, unlike Huntington's chorea, is primarily a disease of childhood. While it does not necessarily shorten the life span, its crippling and disabling effects certainly eliminate many patients from propagation, because these events usually occur before or at puberty. Indeed, most of the so-called sporadic cases reported in the literature had no family of their own.

The fact that other members of the sibships remained unaffected could then be explained on the basis of a mutation.

Secondly, it can be safely assumed that most of the *formes frustes* go unnoticed so that the carrier parent is usually considered to be normal. Thus, the high incidence of sporadic cases and the relative scarceness of familial instances of idiopathic dystonia neither disprove the hereditary nature of the disease nor do they argue against a prevailing tendency of dominant inheritance.

SUMMARY

Genetic studies have been made on a family in which twelve individuals are affected with idiopathic dystonia musculorum deformans. This condition has been traced through four generations and is inherited as a Mendelian dominant trait with complete penetrance, but with very variable expressivity.

A review of 29 instances of familial occurrence of the condition, reported in the literature, reveals that the disease is most frequently transmitted by a dominant gene, although a recessive mode of inheritance may exist.

The importance of having presented new evidence of dystonia musculorum deformans being a heredo-degenerative disease and for its prevention by genetic counseling rests primarily upon the possibility of better delineating and understanding the "*formes frustes*" of this disease. The different clinical manifestations of these abortive forms of dystonia will be described in a separate publication.

ACKNOWLEDGMENT

We are indebted to Madge T. Macklin, M.D. for help in preparing the manuscript and for valuable suggestions.

REFERENCES

ABRAHAMSON, I. 1922. Presentation of case of familial dystonia of Oppenheim. *J. Nerv. Ment. Dis.* 51: 451–454.

BEDER, W. 1926. Quoted from Mankowsky and Czerny.

BEILIN, J. A. 1934/35. Genetische und klinische Analyse des Torsionssyndroms. *Z. Neur.* 152: 126–144.

BENEDEK, L. AND RAKONITZ, E. 1940. Heredopathic combination of congenital deformity of nose and of myoclonic torsiondystonia. *J. Nerv. Ment. Dis.* 91: 608–624.

BERNSTEIN, ST. 1912. Ein Fall von Torsionskrampf. *Wien. klin. Wschr.* 25: 1567–1571.

DAWIDENKOW, S. N. UND SOLOTOWA, N. A. 1921. Eine Familie mit Torsionspasmus. Mitt. d. staatl. Univ. Baku 1: 151–161. (russ). *Zbl. ges. Neur.* 1922/23. 31: 432–433.

DZERSCHINSKY: quoted from Mankowsky and Czerny.

FOSSEY, H. 1922. A case of dystonia musculorum with remarkable familial history. *New York M. J.* 116: 329–330.

GOWERS, W. R. 1893. *A Manual of Diseases of the Nervous System.* Philadelphia, P. Blakiston.

HAENISCH, 1914. Progressiver Torsionspasmus. *Neur. Cbl.* 33: 69–70.

HALLERVORDEN, J. 1957. Die Torsionsdystonie. Der Hemiballismus. In Henke-Lubarsch: *Handb. d. spz. path. Anat. Hist.* Vol. 13/1: 925–933, Springer-Verlag, Berlin-Göttingen-Heidelberg.

HERZ, E. 1944. Dystonia. II. Clinical Classification. *Arch. Neur. Psychiat.* 51: 319–355.

JAKOB, A. 1924. quoted from Mendel (1936).

JANKOWSKA, H. 1934. Beitrag zur Heredität der Torsionsdystonie. *Neur. polska* 15/17: 258–264. (pol.) *Zbl. ges. Neur.* 74: 359, 1934/35.

KEHRER, F. 1936. Erbliche organische Nervenkrankheiten. Allgemeine Einleitung. In Bumke-Foerster: *Handb. d. Neurologie* Vol. 16: 222–272, Springer Berlin.

KEYSERLINGK, H. VON, 1956. Zum familiären Vorkommen der idiopathischen Torsionsdystonie. *Nervenarzt* 27: 34–35.

MANKOWSKY, B. N. UND CZERNY, L. I. 1929. Zur Frage über die Heridität der Torsionsdystonie. *Mschr. Psychiat.* 72: 165–179.

MENDEL, K. 1919. Torsionsdystonie. (Dystonia musculorum deformans, Torsionsspasmus) *Mschr. Psychiat.* 46: 309–361.

MENDEL, K. 1936. Torsionsdystonie. In Bumke-Foerster: *Handb. d. Neurologie* 16: 848–873. Berlin: Springer.

MUNCH-PETERSEN, C. J. 1930. Studien über erbliche Nervenkrankheiten des Zentralnervensystems. Fälle von hereditärem striärem Symptomenkomplex. *Acta psych. scand.* 5: 493–508.

OPPENHEIM, H. 1911. Über eine eigenartige Krampfkrankheit des kindlichen und jugendlichen Alters (Dysbasia lordotica progressiva, Dystonia musculorum deformans). *Neurol. Cbl.* 30: 1090–1107.

OPPENHEIM, H. 1923. *Lehrbuch der Nervenkrankheiten* 7. Aufl. Berlin, Karger.

QUANDT, J. 1956. Beitrag zur Histopathologie der idiopathischen Torsionsdystonie. *D. Ztschr. Nervenheilk.* 175: 100–108.

PRICE, G. E. 1921. The simultaneous occurrence of dystonia lenticularis in twins: *Arch. Neur. Psychiat.* 5: 768–769.

REGENSBURG, J. 1930. Zur Klinik des hereditären torsionsdystonischen Symptomenkomplexes. *Mschr. Psychiat.* 75: 323–345.

ROSE, A. 1937. quoted from Hallervorden.

SANTANGELO, G. 1934. Contributo clinico alla conoscenza delle forme familiari della dysbasia lordotica progressiva. *Gior. Psychiat.* 62: 52–77.

SCHWALBE, W. 1908. Eine eigentümliche tonische Krampfform mit hysterischen Symptomen. *Inaug. Diss.*

SPILLER, W. G. 1913. A case of dystonia musculorum deformans. *J. Nerv. Ment. Dis.* 40: 529–530.

ŠVEJCAR, J. 1924. Familiäre Krampfleiden. Časopis lék. čes. 63: 97–101. *Zbl. Neur.* 1924, 37: 40.

TAYLOR, E. W. 1920. Dystonia lenticularis (Dystonia musculorum deformans). *Arch. Neur. Psychiat.* 4: 417–427.

VAN BOGAERT, L. 1940/41. Etudes anatomo-cliniques de syndrômes hypercinétiques complexes. *Mschr. Psychiat.* 103: 321–342.

VAN BOGAERT, L. 1946. Aspects cliniques et pathologiques des atrophies pallidales et pallido-luysiennes progressives. *J. Neur.* 9: 125–157.

VAN BOGAERT, L. 1948. Maladies nerveuses systématisées et problèmes de l'hérédité. *Acta neur. psychiatr.* 48: 3–64.

VOGT, C. UND O. 1942. quoted from Hallervorden.

WECHSLER, I. S. AND BROCK, S. 1922. Dystonia musculorum deformans. With special reference to a myostatic form and the occurrence of decerebrate rigidity phenomena. *Arch. Neur. Psychiat.* 8: 538–552.

BOOK REVIEWS

Heredity and Evolution in Human Populations

By L. C. DUNN. Cambridge, Mass., Harvard University Press, 1959. 157 + viii pp., 11 Figs., $3.50.

THIS is the first in a new series of "Harvard Books in Biology," which is announced as designed to provide books written by outstanding authorities for laymen and for scientists whose fields are not specifically biology.

A better beginning for the series could hardly be imagined, and the Editorial Committee for the Series is to be congratulated on their choice. Prof. Dunn is not only one of our leading geneticists, director of the Institute for the Study of Human Variation, but also one of the researchers, unfortunately all too rare, capable of consistently expressing lucid thoughts in lucid English. The result is a book which is a pleasure to read.

Within the confines of 152 small pages of text Prof. Dunn, starting with a short chapter (1) variety, manages to explain clearly and well (2) the principles of heredity as applied to human populations, (3) the methods of evolutionary change in human populations, (4) genes and evolution, (5) race formation, (6) isolated populations and small communities, and to give us (7) a look ahead. These 7 chapters are illustrated by 11 figures.

As would be expected the exposition of genetic and evolutionary mechanisms, in spite of the limitations of space and relative nontechnicality of language, are excellent. And the account of isolated populations, where the author draws on his experience with the Jewish ghetto of Rome, is fascinating.

The book is well printed on good paper and has an index.

WILLIAM C. BOYD
Medical School
Boston University

The Fertility of American Women

By WILSON H. GRABILL, CLYDE V. KISER, AND PASCAL K. WHELPTON. New York: John Wiley and Sons. London: Chapman and Hall. 1958. xvi + 448 pp. $9.50.

BECAUSE it contains such a wealth of detailed information on fertility, this book should be described rather than reviewed. At the outset it may be said that it contains facts and interpretations of interest to almost everyone interested in any aspect of human fertility. At the same time, because it draws from the entire fertility data of the United States, the non-specialist reader will probably restrict his reading to a few chapters closest to his own interests. The authors are recognized experts in the study of fertility and their collaboration insures a high standard of authority.

In essence, *The Fertility of American Women* is a joint summary and interpretation of data primarily from the national censuses of 1910, 1940, and 1950, but including other data, earlier and later, from smaller surveys. The approach is therefore largely determined

by the measures of fertility (absolute numbers of births, age-specific birth rates, number of children under five years, number of children ever born, etc.) and the attributes of the women (age, color, residence, marital status, occupation of husband, education, rental value of dwelling, etc.) used in the census. The variation of fertility by single or multiple attributes is examined in considerable detail. The data presented are usually at the national level and may be adequate in themselves for many readers. The reader unfamiliar with the U. S. census fertility data should note, however, that many of the national fertility distributions presented in this book are also available for regions and individual states in the regular census publications. The particular contribution of this book is its analysis and interpretation of the more general aspects of the census data.

Although oriented toward the demographer and sociologist, this book has valuable data for the human geneticist. Our interest follows necessarily from the fact that fertility, together with viability, determines biological fitness. The particular value of the U. S. Census fertility data is the information given on number of liveborn children ever born per woman of specified age, color, marital status, and residence.

As geneticists, we must note a major shortcoming, for our purposes, of the available census fertility data. It is, simply, that overall biological fitness (viability from birth to adulthood and fertility once adult) cannot be determined from such data. This overall fitness is perhaps best measured by the mean number of liveborn children ever to be born per newborn liveborn individual. Census data, instead, give mean number of livebirths per woman surviving to age x. With the use of life tables or the tables in the monograph *Cohort Fertility* (by Whelpton) it is possible to approximate this measure of fitness closely enough for some purposes.

This book may have its greatest value to human genetics as a guide to the extensive fertility data now available. Although very well supplied with tables, it will not, for many geneticists, obviate the need to go to the original census data. Its stated goal—to analyze census data—is successfully reached and we should ask no more of a single volume.

T. Edward Reed
Department of Human Genetics
University of Michigan Medical School

Perspectives in Marine Biology

Edited by A. A. Buzzati-Traverso. 1958. University of California Press. xiii + 621 pages. $10.

In the spring of 1956 nearly 200 biologists met at the Scripps Institute of Oceanography in La Jolla, California. The purpose of this meeting, which was supported by the Rockefeller Foundation, was to ascertain how the problems of the sea could be attacked with the experimental methods developed largely for studying terrestrial organisms.

The published results of the meetings are divided into four sections: ecology, physiology and biochemistry, behavior, genetics and evolution. Eleven papers are included in the fourth section. As a group they emphasize the fact that the genetics of marine organisms is a science of the future. Perhaps the most difficult problem of all in studying the genetics of marine organisms is that of culture. Ray discusses this at length and suggests some rules for selecting suitable species and lists some species that seem promising. There is no reason to suppose, with the data now at hand, that there are genetic systems peculiar to marine organisms but Lewin, Barigozzi, Yonge, and Montalenti suggest species and situations that should be investigated.

Problems of geographic variation and speciation are dealt with by Loosanoff, Wieser, and Kohn. For the most part the data are interpreted in terms of patterns of speciation known for terrestrial organisms.

Several of the authors make the valid point that our understanding of biology as a whole will be incomplete so long as we are largely ignorant of the biology of marine organisms. Perhaps this conference will stimulate studies that will begin to correct this deficiency.

JOHN A. MOORE
Department of Zoology
Columbia University

Hemophilia B: Genetics, Hematology and Clinical Aspects

By J. K. MOOR-JANKOWSKI, H. J. HUSER, S. ROSIN, G. TRUOG, MARIA SCHNEEBERGER, AND M. GEIGER. Basel (Switzerland): S. Karger, 1958.

HEMOPHILIA B is a study of the inheritance of Christmas disease among the inhabitants of the town of Tenna in Switzerland. The stability of the population of this community and the careful records which were available provided an excellent basis for this type of investigation. As a result, Moor-Jankowski and his associates have accumulated a massive amount of data which, in general, support views widely held by persons familiar with coagulative disorders. Christmas disease in these individuals is moderately severe. Although the defect, as measured *in vitro*, was relatively constant from individual to individual, there was greater variation in its clinical manifestations. The authors attribute these variations to hereditary differences in constitution, although it seems more likely that the physical buffets each of us suffers from the outside world differ from person to person. Similarly, the abatement in severity with age which is reported may reflect in part the greater care with which we all protect ourselves against injury as we move through the years.

The pattern of heredity described conforms to the accepted view that Christmas disease is due to a sex-linked recessive gene. As in classic hemophilia, the heterozygous carriers neither bled nor had detectable abnormalities. One exception was a potential carrier in whom the prothrombin consumption test was abnormal. The accumulated data indicate that the female carriers of Christmas disease tended to have more children than non-carriers. One hesitates to draw a moral from this observation.

It is unfortunate that the authors have felt it necessary to use the term hemophilia B, with its implication that the disorder is identical except in some detail with hemophilia. That generalized disorder of coagulationists, nomenclaturopathy, seems to have spread to the geneticists.

This sort of study is most useful in providing a firm basis for views otherwise derived from small samples of the population. One can only congratulate the authors on their tenacity.

OSCAR D. RATNOFF
Department of Medicine
Western Reserve University

Looking at Chromosomes

By JOHN MCLEISH AND BRIAN SNOAD. New York. St. Martin's Press. 1958. pp. 87, $3.75.

THE behavior patterns of mitotic and meiotic chromosomes of a single plant species, *Lilium regale*, are recorded, with considerable clarity, by means of the Feulgen squash method. The authors fulfill their aim to provide a continuous and uninterrupted series of excellent photographs of the various phases of mitosis and meiosis. Attention is given to mitosis in the root meristem, and the divisions preceding the formation of pollen grains and embryo sacs. Definitions of terms are complete and to the point, with appropriate references to 18 generalized line drawings and 39 photographs of dividing cells and nuclei. Chromosome synapsis, chiasma formation and genic segregation, centromeric division, pollen tube germination, and fertilization are but a few of the phenomena considered in the text and drawings.

Although the authors intend this to be only an introduction to chromosome behavior, the addition of a number of salient features would have resulted in making this a valuable reference. The absence of cytoplasmic details (spindle) in the photographs, the omission of experiences pertaining to the Feulgen staining procedure, the vagueness of the time element regarding synchronization of division stages during meiosis and the formation of pollen grains and embryo sacs, and a number of unmentioned photographic factors are expected to leave the interested students, whom the authors, "urge. . . to make some [slides and photographs] for themselves," desirous for more specific technical citations.

The alert student, under these circumstances, may consider this small book expensive for his budget; a lecturer, however, will find it an excellent guide from which to prepare a detailed but simple outline on the basic similarities and differences between mitosis and meiosis, as well as to supplement laboratory demonstrations using commercially prepared slides of similar liliacious plants.

GEORGE YERGANIAN
Children's Cancer Research Foundation, Inc.
Boston 15, Massachusetts

Safe Handling of Radioisotopes

By INTERNATIONAL ATOMIC ENERGY AGENCY. Vienna, Austria, 1958, pp. 99. Available in the U. S. through International Publications, Inc., 801 Third Avenue, New York 22.

THIS is the first in a proposed series of monographs to be published by the International Atomic Energy Agency under the general title, Safety Series. The Agency is composed of over sixty member nations, including the U. S.

The publication provides authoritative and detailed information concerning the safe handling of radioisotopes and should be of value to all those who use radioisotopes.

Heredity Counseling

Edited by HELEN G. HAMMONS. New York: Paul B. Hoeber, Inc. pp. 112. $4.00.

THIS little book contains the papers presented by seventeen discussants at a symposium sponsored by the American Eugenics Society in 1958. It should serve as a useful antidote to articles and books of the "do it yourself" or cookbook variety. The first paper, by Josef Warkany, is especially well done and has long been needed.

Physicians and others who are not genetically trained but have patients or clients who require genetic counseling will find this book very useful as a guide to the Genetic Counselor.

BIBLIOGRAPHY OF HUMAN GENETICS*

R. H. POST

Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan

Selections from the *Current List of Medical Literature* through February, 1959 and other sources

296. AGER, J. A., LEHMANN, H., & VELLA, F. 1958. Haemoglobin Norfolk: a new haemoglobin found in an English family with observations on the naming of new haemoglobin variants. *Brit. M. J.* 5095: 539–541.
297. ALEXANDER, J. O., & GRANT, P. W. 1958. Monilethrix; report of three cases with family history. *Scot. M. J.* 3(8): 356–360.
298. ALLAN, J. D., CUSWORTH, D. C., DENT, C. E., & WILSON, V. K. 1958. A disease, probably hereditary, characterized by severe mental deficiency and a constant gross abnormality of aminoacid metabolism. *Lancet*, Lond. 1(7013): 182–187.
299. ALLISON, A. C. 1958. The genetical and clinical significance of the haptoglobins. *Proc. R. Soc. M.*, Lond. 51(8): 641–645.
300. ALLISON, A. C., & BLUMBERG, B. S. 1958. Familial osteoarthropathy of the fingers. *J. Bone Surg. Brit. Vol.* 40-B(3): 538–545.
301. ALLORI, L., & VITAMIA, P. 1958. Osservazioni genetistiche su 44 casi di cardiopatie acquisite. [Genetic observations on 44 cases of acquired heart disease] *Acta genet. med. gemellol.*, Roma 7(3): 397–410.
302. AMYOT, R. 1958. L'Audi-mutité; document clinique portant sur trios membres d'une même fratrie. [Audimutitas; clinical observation on three members of the same family] *Presse méd.* 66(56): 1289–1292.
303. ANASTASI, A. 1958. Heredity, environment, and the question how? *Psychol. Rev.* 65(4): 197–208.
304. ANDERSEN, J. 1958. Modifying influence of the secretor gene on the development of the ABH substance; a contribution to the conception of the Lewis group system. *Vox sanguinis*, Basel 3(4): 251–261.
305. ANONYMOUS. 1958. Cystinuria. *Q. Rev. Pediat.* 13(3): 113-115.
306. ANONYMOUS. 1958. Inheritance of rheumatoid arthritis. *Brit. M. J.* 5101: 903.
307. ANONYMOUS. 1958. Marfan's syndrome. *Brit. M. J.* 5101: 903–904.
308. ANONYMOUS. 1958. Peptic ulcer in twins. *Brit. M. J.* 5085: 1470–1471.
309. ARONSON, S. M., SAIFER, A., KANOF, A., & VOLK, B. W. 1958. Progression of amaurotic family idiocy as reflected by serum and cerebrospinal fluid changes. *Am. J. Med.* 24(3): 390–401.
310. BEARN, A. G., & FRANKLIN, E. C. 1958. Some genetical implications of physical studies of human haptoglobins. *Science* 128 (3324): 596–597.
311. BEKER, S., & READ, A. E. 1958. Familial Dubin-Johnson syndrome. *Gastroenterology* 35(4): 387–389.
312. BERNARD, J., MATHÉ, G., & NAJEAN, Y. 1958. Contribution à l'étude clinique et physiopathologique de la maladie de Fanconi. [Clinical & physiopathological study of Fanconi's disease] *Rev. fr. clin. biol.* 3(6): 599–612.
313. BETKE, K. 1958. Hämoglobinanomalien. *Schweiz. med. Wschr.* 88(41): 1005–1100.
314. BOLIA, M. C., & ROVESCALLI, A. 1958. Indagini istochimiche ed osservazioni anatomopatologiche in due forme di distrofia corneale familiare. [Histochemical data and anatomopathological observations in two forms of familial corneal dystrophy] *Ann. ottalm.* 84(6): 267–290.
315. BRANDT, H., & REVAZ, C. 1958. Jumelles univitellines et situs inversus. [Monozygotic twins and situs inversus] *J. génét. humaine*, Genève 7(1–2): 33–45.
316. CARP, C. 1958. Disociatie atrio-ventriculară completă, congenitală şi familială la patru membri ai aceleiaşi familii. [Complete, congenital and familial auriculoventricular dissociation in four members of the same family] *Med. int.*, Bucur. 10(5): 761–768.

* Supported in part by the National Institutes of Health, grant number C-3874.

317. Chaudhuri, A., Chaudhuri, J. N., & Chaudhuri, K. C. 1958. Congenital porphyria in siblings; with a brief review of congenital and hereditary porphyrias. *Ind. J. Pediat.* 25(123): 157–172.
318. Creveld, S. Van, & Liem, K. H. 1958. Congenital afibrinogenemia. *Ét. néonatal.* 7(3): 89–100.
319. Dailly, R., & Samson, M. 1958. Forme familiale d'arthrogrypose. *Rev. neur.*, Par. 98(4): 320–328.
320. Denson, K. W. 1958. Electrophoretic studies of the Prower factor; a blood coagulation factor which differs from factor VII. *Brit. J. Haemat.* 4(3): 313–325.
321. Deutsch, E. 1957. Eine neue Thrombopathie-Familie. [A new familial thrombopathy] *Thromb. diath. haem.*, Stuttg. 1(2): 261-263.
322. Didisheim, P., Ferguson, J. H., & Lewis, J. H. 1958. Hemostatic data in relatives of hemophiliacs A and B; evidence for modifying the classical sex-linked recessive hypothesis. *A. M. A. Arch. Int. M.* 101(2): 347–354.
323. Di Sant'Agnese, P. A., Grossman, H., Darling, R. C., & Denning, C. R. Saliva, tears and duodenal contents in cystic fibrosis of the pancreas. *Pediatrics* 22(3): 507–514.
324. Dorn, H. 1958. Allergische Diathese in einer Familie über vier Generationen. [Allergic diathesis in a family of four generations] *Zschr. Haut & Geschlkr.* 25(2): 45–48.
325. Dorn, H. 1957. Biochemisch-genetische Betrachtungen zur Epidermolysis bullosa hereditaria und entsprechende therapeutische Massnahmen. [Biochemical-genetic notes on hereditary epidermolysis bullosa and adequate therapeutic measures] *Zschr. Haut & Geschlkr.* 23(11): 316–320.
326. Driscoll, S. G., & Hsia, D. Y. Y. 1958. The development of enzyme systems during early infancy. *Pediatrics* 22(4), Part 2: 785–845.
327. Durham, D. G. 1958. Congenital hereditary Horner's syndrome. *A. M. A. Arch. Ophth.* 60(5): 939–940.
328. Eberlein, W. R., & Bongiovanni, A. M. 1956. Congenital adrenal hyperplasia: an inborn error of metabolism. *Helvet. paediat. acta* 11(2): 105–117.
329. Ehring, F., & Niermann, H. 1958. Haut- und Halslymphknotentuberkulose bei 88 Zwillingspaaren. [Cutaneous and cervical lymph node tuberculosis in 88 pairs of twins] *Arch. klin. exp. Derm.*, Berl. 205(6): 617–627.
330. Enger, E. 1958. Wilson's sykdom, spesielt med henblikk på de biokjemiske forstyrrelser. [Wilson's disease with special reference to biochemical disorders] *Tskr. Norske laegeforen.* 78(16): 718–784.
331. Erdbrink, W. L. 1957. Ocular myopathy associated with retinitis pigmentosa. *A. M. A. Arch. Ophth.* 57(3): 335–338.
332. Fowler, E. P. 1958. Otosclerosis in ten pairs of identical twins; a continuing study. *Ann. Otol. Rhinol.* 67(3): 889–904.
333. Furusho, T. 1957. A genetic study on congenital deafness. *Japanese J. Hu. Gen.* 2(2): 35–58.
334. Gayral, L., & Gayral, J. 1958. Le faciès discoïde et le status rhizomégalo-discoïde; étude génétique. [Discoid face and rhizomegal-discoid status; genetic study] *J. génét. humaine*, Genève 7(1–2): 46–66.
335. Geisler, E., & Ströder, J. 1958. Zur Problematik der Phenylbrenztraubensäure-Oligophrenie. [Problem of phenylpyruvic oligophrenia] *Ann. paediat.*, Basel 191(3): 147–174.
336. Gerald, P. S., & Diamond, L. K. 1958. A new hereditary hemoglobinopathy (the Lepore trait) and its interaction with thalassemia trait. *Blood*, Bost. 13(9): 835–844.
337. Gérard-Lefebvre, Benoit, M., & Quéval, B. 1958. Polycorie glycogénique hépatique chez trois enfants d'une même fratrie. [Glycogenic hepatic polycoria in 3 children of the same stock] *Arch. fr. pédiat.* 15(3): 388–395.
338. Gibson, D., & Gibbins, R. J. 1958. The relation of mongolism stigmata to intellectual status. *Am. J. Ment. Defic.* 63(2): 345–348.
339. Gold, E. R., Ikin, E. W., & Mourant, A. E. 1958. Serum containing three Rh autohaemagglutinins. *Brit. M. J.* 5107: 1273–1274.
340. Goldenberg, I. S., & Hayes, M. A. 1958. Breast carcinoma and ABO blood groups. *Cancer*, Phila. 11(5): 973–974.

341. Goldschmidt, R. B. 1958. Materials for the study of dominant personality traits. *Folia hered. path.* 2(4): 267–295.
342. Gorer, P. A. 1958. Genetics of human cancer: a general survey of methods. *Ann. N. York. Acad. Sc.* 71(6): 1189–1197.
343. Gorsuch, G. E. 1958. Essential hyperlipemia. *U. S. Armed Forces M. J.* 9(10): 1414:1424.
344. Graham, S., & Lilienfeld, A. M. 1958. Genetic studies of gastric cancer in humans: an appraisal. *Cancer*, Phila. 11(5): 945–958.
345. Greig, H. B., Metz, J., Bradlow, B. A., Theron, J. J., & Morris, R. W. 1958. The familial crisis in hereditary spherocytosis: report of five cases. *S. Afr. J. M. Sc.* 23(1): 17–32.
346. Greither, A. 1958. Über eine mit Keratosen und Pigmentstörungen einhergehende erbliche Dysplasie der Haut. [A hereditary dysplasia of the skin associated with keratoses and pigment disorders] *Hautarzt* 9(8) 364–369.
347. Hackzell, G., & Lundmark, C. 1958. Familial juvenile nephronophthisis. *Acta paediat.*, Upps. 47(4): 428–440.
348. Hahneman, B. M., & Alt, H. L. 1958. Cyclic neutropenia in a father and daughter. *J. Am. M. Ass.* 168(3): 270–272.
349. Hallgren, B. 1958. Speciella läs- och skrivsvårigheter (s.k. ordblindhet). II. Genetiska synpunkter. [Difficulties in reading and writing (word blindness). II. Genetic aspects] *Nord. med.* 59(15): 516–518.
350. Hara, H. J. 1957. Scleroma; observations on seven cases in one family for 10 years and another case which developed intracranial complications after five years. *Tr. Am. Lar. Rhin. Otol. Soc.:* 326–343.
351. Hargreaves, G. K., & Hellier, F. F. 1958. The association of psoriasis with blood groups. *A. M. A. Arch. Derm.* 78(4): 438–439.
352. Harris, H., Robson, E. B., & Siniscalco, M. 1958. β-Globumin variants in man, *Nature*, Lond. 182(4633): 452.
353. Harrison, H. E. 1958. The Fanconi syndrome. *J. Chronic Dis.* 7(4): 346–355.
354. Hart, M. Van Der, Veer, M. Van Der, Loghem, J. J. Van, Sanger, R., & Race, R. R. 1958. Vr, an antigen belonging to the MNSs blood group system. *Vox sanguinis*, Basel 3(4): 261–265.
355. Havener, W. H., Stine, G. T., & Weiss, L. L. 1958. Corneal donor selection by blood type. *A. M. A. Arch. Ophth.* 60(3): 443–447.
356. Hemmeler, G. 1958. Thrombopathie familiale. *Schweiz. med. Wschr.* 88(41): 1018–1019.
357. Hermann, P. 1958. Le syndrome microphthalmie-rétinite pigmentaire-glaucome. *Arch. opht.*, Par. 18(1): 17–24.
358. Hitzig, W. H., & Zollinger, W. 1958. Kongenitaler Faktor- VII-Mangel; Familienuntersuchung und physiologische Studien über den Faktor VII. [Congenital factor VII deficiency; family investigation and physiological studies on factor VII] *Helvet. paediat. acta* 13(3): 189–203.
359. Holt, S. B. 1958. Genetics of dermal ridges; the relation between total ridge-count and the variability of counts from finger to finger. *Ann. Human Genet.*, London 22(4): 323–339.
360. Houghton, E. A., & Naish, J. M. 1958. Familial ulcerative colitis and ileitis. *Gastroenterologia*, Basel 89(2): 65–74.
361. Howel-Evans, W., McConnell, R. B., Clarke, C. A., & Sheppard, P. M. 1958. Carcinoma of the oesophagus with keratosis palmaris et plantaris (tylosis): a study of two families. *Q. J. Med.*, Oxf. 27(107): 413–429.
362. Hsia, D. Y. Y. 1958. Phenylketonuria: the phenylalanine-tyrosine ratio in the detection of the heterozygous carrier. *J. Ment. Defic. Res.*, Caterham 2(1): 8–16.
363. Hunt, J. A., & Ingram, V. M. 1958. Abnormal human haemoglobins. II. The chymotryptic digestion of the trypsin-resistant core of haemoglobins A and S. *Biochim. biophys. acta*, Amst. 28(3): 546–549.
364. Isaacs, H. 1958. Familial pes cavus, absent tendon jerks and extensive muscular atrophy. *S. Afr. M. J.* 32(33): 830–832.
365. Jankiewicz, H. A., Nadell, M. C., & Benitez, T. A. 1958. A pedigree of arcus juvenilis. *Am. J. Optometr.* 35(1): 3–9.

366. JIM, R. T. 1958. Hemoglobin H disease. *A. M. A. Arch. Int. M.* 102(3): 400–404.
367. JONES, H. I., & DOIG, R. K. 1958. Illness in twins: VI. Sarcoidosis. *Med. J. Australia* 45, Vol. 2(4): 114–117.
368. JÜRGENS, R., LEHMANN, W., WEGELIUS, O., ERIKSSON, A. W., & HIEPLER, E. 1957. Mitteilung über den Mangel an antihämophilem Globulin (Faktor VIII) bei der Aaländischen Thrombopathie (v. Willebrand-Jürgens). [Antihemophilic globulin (factor VIII) deficiency in Aland (Willebrand-Jurgens) thrombopathy] *Thromb. diath. haem.*, Stuttg. 1(2): 257–260.
369. KAELIN, A. 1958. Estimation statistique de la fréquence des tarés en génétique humaine. [Statistical estimation of frequency of hereditary diseases in human genetics] *J. génét. humaine*, Genève 7(1–2): 67–91; cont'd.
370. KEAY, A. J. 1958. The significance of twins in mongolism in the light of new evidence. *J. Ment. Defic. Res.*, Caterham 2(1): 1–7.
371. KLESPER, R., & ACHENBACH, W. 1957. Über eine neue Familie mit Angiohämophilie A (vaskuläre Hämophilie); Hereditäre hämorrhagische Diathese mit verlängerter Blutungszeit und partiellem mangel an Antihämophilem Globulin. [A family with angiohemophilia A (vascular hemophilia); hereditary hemorrhagic diathesis with prolonged bleeding time and partial antihemophilic globulin deficiency] *Thromb. diath. haem.*, Stuttg. 1(2): 223–233.
372. KNOX, W. E. 1958. Sir Archibald Garrod's Inborn Errors of Metabolism. III. Albinism. *Am. J. Human Genet.* 10(3): 249–267.
373. KOCH, G., KRISCHEK, J., & TIWISINA, T. 1957. Beitrag zur Klinik, Pathogenese und Erbpathologie dysontogenetischer (dysraphischer) Störungen des Zentralnervensystems (Septum pellucidum-Cysten, Hirntumor) bei eineiigen Zwillingen. [Clinical course, pathogenesis and heredopathology of dysontogenetic (disraphic) disturbances of the central nervous system, septum pellucidum cysts & brain tumors, in monozygotic twins] *Zschr. menschl. Vererb.* 34(2): 105–123.
374. KOLLER, F. 1958. Physiology and pathology of blood coagulation; a review of the litreature of 1957 (second series). *Thromb. diath. haem.*, Stuttg. 2(3–4): 358–406.
375. KOMAI, T. 1953. A Japanese kindred of hyperphalangism of thumbs and duplication of thumbs and big toes. *Folia hered. path.* 2(4): 307–312.
376. KONUGRES, A., & COOMBS, R. R. 1958. Studies on human anti-A sera with special reference to so-called immune anti-A. *Brit. J. Haemat.* 4(3): 261–269.
377. LEHMANN, H. 1958. Three varieties of human haemoglobin D. *Nature*, Lond. 182(4639): 852–854.
378. LINNEROTH, K., HALLGREN, B., PALMÉN, K., & ZETTERSTROM, R. Primary (genuine) vitamin D resistant rickets. IV. A clinical and genetic study. *Acta paediat.*, Upps. 47(5): 568–592.
379. LIPPMAN, R. W. 1958. The significance of heterozygosity for hereditary metabolic errors related to mental deficiency (oligomentia). *Am. J. Ment. Defic.* 63(2): 320–324.
380. LÖHR, G. W., & WALLER, H. D. 1958. Hämolytische Erythrocytopathie durch Fehlen von Glukose-6-Phosphatdehydrogenase in roten Blutzellen als dominant vererbliche Krankheit. [Hemolytic erythrocytopathy caused by absence of glucose-6-phosphate dehydrogenase in red cells as a dominant hereditary factor] *Klin. Wschr.* 36(18): 865–869.
381. LUKL, P., WIEDERMANN, B., & BARBOŘÍK, M. 1958. Hereditäre Leptocyten-Amänie bei Männern mit Hämochromatose. [Hereditary leptocytic anemia in males with hemochromatosis] *Fol. haemat.*, Frankf. 3(1): 17–45.
382. LUMBROSO, B. D. R. 1958. Disordered eye movements in hereditary spino-cerebellar degeneration. *Rev. neur.*, Par. 98(4): 331.
383. MACMAHON, B., & FOLUSIAK, J. C. 1958. Leukemia and ABO blood group. *Am. J. Human Genet.* 10(3): 287–293.
384. MAHER, J. A., EPSTEIN, F. H., & HAND, E. A. 1958. Xanthomatosis and coronary heart disease; necropsy studies of two affected siblings. *A. M. A. Arch. Int. M.* 102(3): 437–442.
385. MCKUSICK, V. A. 1958. The genetic aspects of cardiovascular diseases. *Ann. Int. M.* 49(3): 556–567.
386. METRAKOS, J. D., METRAKOS, K., & BAXTER, H. 1958. Clefts of the lip and palate in twins; including a discordant pair whose monozygosity was confirmed by skin transplants. *Plastic & Reconstr. Surg.* 22(2): 109–122.

387. Mohn, J. F., Lambert, R. M., Rosamilia, H. G., Wallace, J., Milne, G. R., Moores, P., Sanger, R., & Race, R. R. 1958. On the relationship of the blood group antigens Mia and Vw to the MNSs system. *Am. J. Human Genet.* 10(3): 276–286.
388. Morton, M. E. 1958. Empirical risks in consanguineous marriages: birth weight, gestation time, and measurements of infants. *Am. J. Human Genet.* 10(3): 344–349.
389. Muntendam, H. 1958. Multiple myelomatosis en het syndroom van De Toni-Fanconi. [Multiple myelomas & the De Toni-Fanconi syndrome] *Ned. tschr. geneesk.* 102(35): 1690–1693.
390. Neel, J. V. 1958. Medicine's genetic horizons. *Ann. Int. M.* 49(2): 472–476.
391. Neri Serneri, G. G., & Bartoli, V. 1957. Ricerche genetiche sulla predisposizione all'artrosi dolorosa e sui suoi rapporti con i reumatismi primari. [Genetic research on the predisopsition to painful arthrosis and on their relations to primary rheumatism] *Acta genet. med. gemellol.*, Roma 6(4): 503–521.
392. Novitski, E., & Kimball, A. W. 1958. Birth order, parental ages, and sex of offspring. *Am. J. Human Genet.* 10(3): 268–275.
393. Obrador, S., Lorente, L., Boixadós, J. R., Jiménez-Casado, M., & Pastor, E. M. 1958. Craneoestenosis en gemelos. [Craniostenosis in twins] *Rev. clin españ.* 70(1): 46–47.
394. Okajima, M. 1958. Probability of paternity in Rh blood groups. *Acta genet. med. gemellol.*, Roma 7(3): 321–360.
395. Oliver, C. P. 1958. Studies on human cancer families. *Ann. N. York Acad. Sc.* 71(6): 1198–1212.
396. Osborne, R. H., Horowitz, S. L., & De George, F. V. 1958. Genetic variation in tooth dimensions: a twin study of the permanent anterior teeth. *Am. J. Human Genet.* 10(3): 350–356.
397. Pauling, L. 1958. Genetic and somatic effects of carbon-14. *Science* 128(3333): 1183–1186.
398. Perkoff, G. T., Nugent, C. A. Jr., Dolowitz, D. A., Stephens, F. E., Carnes, W. H., & Tyler, F. H. 1958. A follow-up study of hereditary chronic nephritis. *A. M. A. Arch. Int. M.* 102(5): 733–746.
399. Pescetto, G., 1957. Rilievi patologici precoci nelle manifestazioni extrapiramidali del morbo di Parkinson; loro applicazione nello studio della trasmissione ereditaria della malattia. [Early pathological data on extrapyramidal manifestations of Parkinson's disease; their use in study of the hereditary transmission of the disease] *Riv. pat. nerv.* 78(3): 1165–1168.
400. Petersen, H., & Wolff, H. 1957. Über das kombinierte Auftreten mehrerer Krankheiten bei 7 Geschwistern; kleinwuchs, Hypogenitalismus, Diabetes mellitus, Dystrophia musculorum progressiva, Schwachsinn, Epilepsie, kongenitale Hüftgelenksluxation und akute lymphatische Leukämie. [Combined occurrence of several diseases in 7 siblings; retarded growth, hypogenitalism, diabetes mellitus, progressive muscular dystrophy, mental retardation, epilepsy, congenital pelvic luxation and acute lymphatic leukemia] *Zschr. menschl. Vererb.* 34(2): 237–249.
401. Poli, M. 1955. Néphropathie médicale bilatérale familiale à évolution chronique. [Familial chronic bilateral kidney disease] *Helvet. med. acta* 22(2): 109–122.
402. Rapaport, H. G. 1958. Heredity and allergy. *N. York State J. M.* 58(3): 393–396.
403. Redies, H., Quenzer, K., Tolksdorf, M., Saile, M. L., & Wiedemann, H. R. 1958. Über die Häufigkeit der Pelger-Anomalie. [Incidence of Pelger's nuclear anomaly] *Schweiz. med. Wschr.* 88 (41): 1002–1003.
404. Reemtsma, K., Di Sant'Agnese, P. A., Malm, J. R., & Barker, H. G. 1958. Cystic fibrosis of the pancreas: intestinal absorption of fat and fatty acid labeled with I-131. *Pediatrics* 22(3): 525–532.
405. Reimann, H. A., McKechnie, W. G., & Stanisavljevic, S. 1958. Hereditary sensory radicular neuropathy and other defects in a large family: reinvestigation after twenty years and report of a necropsy. *Am. J. Med.* 25(4): 573–579.
406. Roberts, J. A. 1958. Gruppi sanguigni e suscettibilità alle malattie. [Blood groups & susceptibility to disease] *Recenti progr. med.*, Roma 25(1): 1–39.
407. Robinson, A. W., Henry, C. L., Arms, A. V., & Dodge, M. 1958. Leriche syndrome; report of two cases occurring in brothers. *J. Kansas M. Soc.* 59(10): 437–439.
408. Rösler, H. D. 1958. Über die Vererbung der Finger längenproportion. [Hereditary transmission of finger length ratios] *Acta genet. med. gemellol.*, Roma 7(3): 361–382.
409. Sanghvi, L. D., Sukumaran, P. K., & Lehmann, H., 1958. Haemoglobin J trait in two Indian women, associated with thalassaemia in one. *Brit. M. J.* 5100: 828–830.

410. SCHNEIDER, R. G., & HAGGARD, M. E. 1958. Haemoglobin P (the Galveston type). *Nature*, Lond. 182(4631): 322–323.
411. SCHULL, W. J. 1958. Empirical risks in consanguineous marriages: sex ratio, malformation, and viability. *Am. J. Human Genet.* 10(3): 294–343.
412. SMITH, W. G. 1958. Multiple polyposis, Gardner's syndrome and desmoid tumors. *Dis. Colon Rectum* 1(5): 323–332.
413. SOINI, A., & SERRA, A. 1958. Ricerche sulla familiarità del carcinoma ovarico. I. Osservazioni sui casi primari e rilievi sull'età di manifestazione dei carcinomi ovarici e sulla fertilità delle pazienti affette da cancro dell'ovaio. [Research on the heredity of ovarian carcinoma. I. Observations on primary cases and data on the age at onset of ovarian carcinomas and on the fertility of patients with ovarian cancer] *Acta genet. med. gemellol.*, Roma 7(3): 383–396.
414. SOULIER, J. P., PROU-WARTELLE, O., WEILLAND, C., & MÉNACHÉ, D. 1958. Déficit congénital en proaccélérine (facteur V); quelques données nouvelles. [Congenital proaccelerin deficiency (factor V); various new data] *Thromb. diath. haem.*, Stuttg. 2(3–4): 250–268.
415. STARK, P. 1958. Étude clinique et génétique d'une famille atteinte d'atrophie musculaire progressive neurale (amyotrophie de Charcot-Marie). [Clinical and genetic study of a family with progressive neuropathic muscular atrophy (Charcot-Marie amyotrophy)] *J. génét. humaine*, Genéve 7(1–2): 1–32.
416. STECHER, R. M. 1957. Heredity in joint diseases: the genetics of Heberden's nodes, rheumatoid arthritis, ankylosing spondylitis, rheumatic fever, gout, and osteoarthritis of the hip. *Docum. rheumat. Engl. Ed.*: 5–74.
417. STEPHENS, F. E., GARDNER, E. J., & WOOLF, C. M. 1958. A recheck of kindred 107, which has shown a high frequency of breast cancer. *Cancer*, Phila. 11(5): 967–972.
418. STERN, K. 1958. The ratio of monozygotic to dizygotic affected twins and the frequencies of affected twins in unselected data. *Acta genet. med. gemellol.*, Roma 7(3): 313–320.
419. STRAATEN, A. VAN. 1958. Mongoloïde idiotie, een genetisch problem? [Mongolian idiocy as a genetic problem] *Ned. tschr. geneesk.* 101(52): 8–14.
420. SUTNICK, A. I., & DYKE, R. W. 1958. Constitutional hyperbilirubinemia (Gilbert's disease): case report and review. *J. Indiana M. Ass.* 51(11): 1544–1547.
421. SUTTER, J. 1958. Recherches sur les effets de la consanguinité chez l'homme. *Biol. méd.*, Par. 47(5): 563–660.
422. SUTTER, J., & TRAN-NGOC-TOAN. 1957. The problem of the structure of isolates and of their evolution among human populations. *Sympos. Quant. Biol.* 22: 379–383.
423. SZEINBERG, A., SHEBA, C., & ADAM, A. 1958. Selective occurrence of glutathione instability in red blood corpuscles of the various Jewish tribes. *Blood*, Bost. 13(11): 1043–1053.
424. TORRIOLI RIGGIO, G. 1958. Considerazioni su una famiglia di granulopenici. [Considerations on a family of granulopenics] *Acta genet. med. gemellol.*, Roma 7(2): 237–248.
425. UEHLINGER, E. 1957. Pathologische Anatomie der chondro-ektodermalen Dysplasie Ellis-van Creveld. [Pathological anatomy of chondro-ectodermal dysplasia of Ellis-van Creveld] *Schweiz. Zschr. allg. Path.* 20(6): 754–766.
426. UNDRITZ, E. 1958. Die Chediak-Steinbrinck-Anomalie oder erblichkonstitutionelle Riesengranulation (Granulagiganten) der Leukocyten. [Chediak-Steinbrinck anomaly or hereditary-constitutional giant granulation of leukocytes] *Schweiz. med. Wschr.* 88(41): 996–999.
427. VEALE, A. M. 1958. Possible autosomal linkage in man. *Nature*, Lond. 182(4632): 409–410.
428. VELLA, F., AGER, J. A., & LEHMANN, H. 1958. An abnormal haemoglobin in a Chinese: haemoglobin G. *Nature*, Lond. 182(4633): 460–461.
429. VERSO, M. L., & SIMMONS, R. T. 1958. The second Australian example of anti-S associated with the MNSs blood groups. *Med. J. Australia* 45, Vol. 2(9): 286–289.
430. VRIES, E. DE, VAN BOGAERT, L., & EDGAR, G. W. 1958. Nouvelles observations d'idiotie familiale avec dégénérescence spongieuse des centres nerveux (maladie oedémateuse progressive cérébrale de la première enfance). [New observations on familial idiocy with spongy degeneration of nerve centers (progressive edematous cerebral disease of infants)] *Rev. neur.*, Par. 98(4): 271–295.
431. WADLINGTON, W. B. 1958. Familial trembling of the chin. *J. Pediat.*, S. Louis 53(3): 316–321.
432. WALLS, G. L. 1958. Graham's theory of color blindness. *Am. J. Optometr.* 35(9): 449–460.

433. WENT, L. N., & MACIVER, J. E. 1958. An unusual type of hemoglobinopathy resembling sickle cell-thalassemia disease in a Jamaican family. *Blood*, Boston 13(6): 559–568.
434. WIENER, A. S. 1958. Blood-group nomenclature. *Science* 128(3328): 849–850; passim.
435. WOLFF, J. A., MICHAELS, R. H., & VON HOFE, F. H. 1958. Hemoglobin H-thalassemia disease. *Blood*, Boston 13(5): 492–501.
436. WOOLF, L. I., GRIFFITHS, R., MONCRIEFF, A., COATES, S., & DILLISTONE, F. 1958. The dietary treatment of phenylketonuria. *Arch. Dis. Childh.* Lond. 33: 31–45.
437. YANG, C., CHIANG, J. C., FENG, P. H., & YANG, N. H. 1958. Color-blindness among the Chinese. *Chin. M. J.* 76(3): 283–284.
438. ZABRISKIE, J., & REISMAN, M. 1958. Marchesani syndrome. *J. Pediat.*, S. Louis 52(2): 158–169.
439. ZETTERSTRÖM, R. 1958. Disseminated lipogranulomatosis (Farber's disease). *Acta paediat.*, Upps. 47(5): 501–510.
440. ZIFF, M., SCHMID, F. R., LEWIS, A. J., & TANNER, M. 1958. Familial occurrence of the rheumatoid factor. *Arthritis & Rhemuat.* 1(5): 392–399.

HE

THE UNIVERSITY OF MICHIGAN
MAY 29 1962
NURSING LIBRARY

THE AMERICAN JOURNAL of

HUMAN GENETICS

VOLUME 11 June 1959 NUMBER 2
PART 2 (OF 2 PARTS)

SYMPOSIUM ON GENETICS IN MEDICAL RESEARCH

Reprinted with permission from Volume 34 Number 6 of
THE JOURNAL OF MEDICAL EDUCATION

Introduction ..... John Z. Bowers 239
Opportunities for Research in Human Genetics ..... James V. Neel 240
The Chromosomes of Man ..... Curt Stern 251
Methodology in Human Genetics ..... Arthur G. Steinberg 313
Importance of Genetics of Viruses in Medical Research ..... Elmer Hammarsten 318
Discussion ..... George K. Hirst 322
Bacterial Genetics and Infectious Disease ..... E. A. D. Stocker 324
Genetic Aspects of Tissue Transplantation and Tolerance ..... Ray D. Owen 340
Discussion ..... W. E. Bishop 351
The Inheritance of "Vascular Hemophilia": A New and Interesting Problem in Human Genetics ..... John B. Graham 353
Introduction to "Panel: Selective Factors in the ABO Polymorphism" ..... William C. Boyd 367
A Possible Example of the Action of Selection in Human Blood Groups ..... William C. Boyd 368
Correlations of ABO Blood Groups with Peptic Ulcer, Cancer, and Other Diseases ..... C. E. Clarke 369
Selection in ABO Polymorphism in Japanese Populations ..... D. Matsunaga 405
The Relation of the ABO and Rh Blood Groups to Differential Reproduction ..... Bernice H. Cohen and Estelle Glass 414
The Protective Action of ABO Incompatibility on Rh Immunization and Rh Hemolytic Disease—Theoretical and Clinical Implications ..... Philip Levine 434
Discussion: Selective Factors in the ABO Polymorphism ..... J. A. Buchanan 449
Comment ..... Ernst Mayr 451
Discussion on Somatic Cell Genetics ..... George Wald 453

Published Quarterly by
THE AMERICAN SOCIETY OF HUMAN GENETICS

THE AMERICAN JOURNAL OF HUMAN GENETICS

is a quarterly record of research, review and bibliographic material relating to heredity in man, and to the applications of genetic principles in medicine, anthropology, psychology, and the social sciences. It is owned and controlled by the American Society of Human Genetics, and is edited by a staff appointed by its Board of Directors.

Editor:

ARTHUR G. STEINBERG
Western Reserve University
Cleveland 6, Ohio

Associate Editors:

F. C. FRASER
McGill University

C. N. HERNDON
Bowman Gray School of Medicine

HORACE W. NORTON
University of Illinois

NEWTON E. MORTON
University of Wisconsin

HERMAN M. SLATIS
Argonne National Laboratory

JOHN B. GRAHAM
University of North Carolina

Advertising Manager: DR. R. H. POST, Quogue, Long Island, N. Y.

Subscription, per volume, $10.00 *Single numbers* $3.00

THE American Journal of Human Genetics is published quarterly at Baltimore, Md. in March, June, September, and December. A volume will consist of four numbers, totaling approximately 400 pages. Subscription and other business communications should be addressed to the Treasurer, Dr. H. Warner Kloepfer, Tulane University, New Orleans, La. Remittance for subscriptions from countries other than the United States must be payable in U. S. currency or its full equivalent. Checks or money orders should be made payable to the American Society of Human Genetics.

Copyright, 1959, by the American Society of Human Genetics. All rights reserved.
Made in United States of America

A SYMPOSIUM ON GENETICS IN MEDICAL RESEARCH

University of Wisconsin, Madison, Wisconsin, April 7–10, 1958

Introduction

The development of Medical Genetics has lagged behind animal and plant genetics, and bacterial genetics. The most rapid growth has occurred during the past 10 years, and some formal teaching has been developed in the curricula of a few medical schools during this period. In 1953, a questionnaire revealed that 55 per cent of the Medical Schools in the United States and Canada gave some instruction in Genetics, but only six offered formal courses.

Research in medical genetics has shown a comparable rapid growth during the past ten years. Genetic implications in the causation and prevention of congenital malformations, metabolic diseases and degenerative phenomena represent fields for continued investigation.

This Symposium on Genetics in Medical Research was initiated from conversations between representatives of the N.I.H. and Wisconsin. The objective was the further expansion of genetics in medical research and education. An effort was made to show that experimental study on various laboratory organisms (animal, bacteria, and other) has the same role in medical genetics as the counterpart does in medical biochemistry and physiology.

Each Medical School in the United States was invited to send a teacher concerned with the development of a medical genetics program in that institution. Distinguished geneticists from the United States and other countries presented discussions around carefully selected topic areas. These principal discussions were followed by small group conferences with a maximum emphasis on individual participation.

The publication of these papers is a departure from the customary program of this Journal. However, because of their great potential significance in medical education (and the fact that the Editor was Chairman of the Planning Committee), it was felt that they should be published here.

JOHN Z. BOWERS, M.D.
Editor-in-Chief

Opportunities for Research in Human Genetics

JAMES V. NEEL, M.D., PH.D.
Department of Human Genetics, University of Michigan Medical School,
Ann Arbor, Mich.

From the position of this paper on the program, as well as the very pleasant postprandial state in which we now find ourselves, I would presume—and have presumed—that my presentation this evening was not intended to be characterized by closely measured logic applied to a very specific problem, but to be more in the nature of a general and perhaps somewhat rambling discourse.

Before getting on to the business at hand, I may, perhaps, be permitted to extend a few words of congratulation to our Wisconsin hosts, not only for the excellence of the program they have arranged, but for all the very significant recent developments here in the field of medical genetics. It is particularly noteworthy that this effort should materialize on a campus so long known for outstanding research in experimental genetics. As a relative "old timer" in the field of human genetics who has taken it upon himself to speak for the other old timers, I want to tell you, in all honesty and sincerity, just how heart-warming it is to see the quickening tempo of emergence, all over the country, of efforts like this one. There are those in this audience—I see Madge Macklin, Herluf Strandskov, and Franz Kallmann, to mention only three—who can remember even better than I the parlous state of human genetics a scant 15 or 20 years ago. The change in the intellectual—and financial—climate is truly staggering. I well recall how, at the time I abandoned Drosophila in favor of man as an object of genetic research, it was considered by many of my friends and associates as a very rash move, on the grounds that not only was it out of the question to do anything significant with human material, but there were just no positions to support the few wishing to work in the field. Today the shortage is clearly of men, not positions, while the subject matter of this Symposium alone seems adequate testimony on the question of significance.

By way of an introduction to my topic, I should point out that there are certain intellectual adjustments that the worker in the field of human genetics must make, especially if he enters the field from the background of experimental plant and animal genetics. He is now working with a material which not only cannot be bred at will but often must be pursued considerable distances, in order to determine the results of such breeding experiments as it may inadvertently have carried out. Even when there is the complete cooperation of the "material," there is this matter of generation time, which puts man in a very select class indeed. In this connection, there is one aspect of the development here at Wisconsin which I cannot resist commenting on. You have here a Department of Medical Genetics, headed by a microbiologist. This is quite different from our own development at Michigan, where we have a Department of Human Genetics, with the work on microbial genetics in the Department of Bacteriology. For myself, I'm just as happy to see it there. Having finally adjusted to the peculiar problems of human genetics, I must confess to still feeling a nostalgic and even envious twinge when I read or listen to a paper concerned with the fantastic manipulations of

the genetic material now possible in some of the micro-organisms. I can stand this twinge occasionally but fear that, were I working in really close proximity to someone like Dr. Lederberg, with day-to-day bulletins—any one of which covered almost as many generations as the whole of recorded human history—the twinges might come so frequently as to leave me in a state of status anginosus. Seriously, I think this close juxtaposition of work on the agent of disease as well as the host is really quite exciting, and a development to be watched with interest.

However, while the study of human genetics has its problems, it also has its advantages. There is an increasing number of questions which can be pursued just as effectively in man as in any other organism; and there is a growing list of questions which can be pursued even *more* effectively in man than in any other mammal. The grand strategy of human genetics lies in recognizing the advantages of man and making the most of them. Tonight I would like to touch upon some of the areas where human genetics can advance not only very rapidly, but with results which should illuminate the genetics of other organisms. If at places this sounds a little like a "pep" talk, then it is no coincidence, because this is what it is meant to be. Without for one moment minimizing all the practical problems with which the student of human genetics is confronted, I should like to emphasize, by means of a series of examples, the manner in which recent developments in medicine, demography, public health, etc., have improved the position of the human geneticist vis-à-vis his "experimental" organism. In point of fact, much of what we are discussing during this Symposium is eloquent testimony to these developments. The particular areas to come under discussion this evening include selected problems in biochemical genetics, in the genetic structure of populations, and in the nature of natural selection. Out of the many examples that could be chosen, I have, on the one hand, deliberately stayed away from areas to be covered by other speakers and, on the other hand, elected to draw rather heavily on some developments with which the group at Michigan has had first-hand experience, simply because this is the material with which I am most familiar. Other pertinent examples of the expanding horizons of human genetics, from the biochemical to the clinical level, will be found in such recent monographs as those of Harris (15) and McKusick (28).

THE STUDY OF NATURAL SELECTION IN MAN

Let us consider first the study of natural selection in man. In the final analysis, natural selection is the result of either differential mortality or differential fertility involving individuals of dissimilar genetic constitutions. That the human species may currently be subject to very significant selective pressures can scarcely be doubted—cf. Fisher (13); Neel (32); Crow (9). Many opportunities exist for the study of these pressures. In an experimental organism, such as the fruit fly, it is a very simple matter to introduce known numbers of individuals of differing genotypes into carefully controlled environments and to determine, after a given number of generations, the proportions in which the original and various derived genotypes are found. From this one can generate very precise statements of the relative reproduction of the different genotypes under these conditions, i.e., the selective value of the genotypes. One can go further, again using Drosophila as an example, and determine at what stage in the life cycle selection occurs, and, to some extent, how this selection takes place. However, many of the same types of observations can all be made in man, with a degree of detail which at least in part offsets the longer generation time. Thus, we certainly are in a better position to analyze the workings of both genetically determined differential mortality and fertility in man than in any other organism, complicated though the latter may be in man by psychic factors with which many organisms apparently do not contend.

Two recent examples will serve to illustrate how one can analyze in man the dynamics of the fertility difference between two genotypes. The first concerns multiple neurofibromatosis (Crowe, Schull, and Neel [10]). Individuals afflicted with this dominantly inherited trait have a reproductive expectancy at birth which is 52.7 per cent that of the normal siblings of sporadic cases of the disease. They are thus subject to rather severe negative selection. This impaired fertility can be analyzed into a number of component parts in a way which illustrates the interplay of physical and psychic components in determining an individual's fertility. To begin with, the disease results in a certain amount of disability and even mortality which clearly interfere with reproduction. For instance, in our series of 223 patients with the disease, there have been nineteen with fatal or incapacitating involvement of the brain or spinal cord prior to age 30, with the final proportion to be higher because many of the individuals in the series are still quite young. Life-expectancy curves for the disease are not available, but there can be no doubt that a diminished life span is a factor in the reduced reproductive expectancy.

There is no evidence that the disease is any more severe in one sex than the other. Nevertheless, there is a rather marked difference between the sexes in their relative fertility—that of males being 41.3 per cent the fertility of the male siblings of sporadic cases; and that of females, 74.8 per cent. In part, this is accounted for by a significantly lower marriage rate of affected males as compared with affected females. We have been unable to decide whether this implies greater discrimination on the part of the female of the species, or a greater social conscience on the part of the male, but at any rate, since the physical effects of the disease appear to be the same in the two sexes, this would seem to be more a sociological than a biological phenomenon. Even after marriage there are fertility differences, however, the married males showing 62.0 per cent of the fertility of the male siblings of sporadic cases, while the females exhibit 88.7 per cent the fertility of these female siblings. To what extent this is biological, and to what extent sociological, would be difficult to say. We have some indications of a lowered sexual drive on the part of affected males, a phenomenon certainly amenable to precise investigation. If this is actually the case, it might also be a factor in the lower marriage rate of affected males. Even were the sex drives of both affected males and females reduced equally, this might be reflected disproportionately in the reproductive performance of the male, because of the more passive role of the female in these matters. At any rate, there is clear evidence for the action of selective factors on at least four levels, with the analysis of the problem still in its early stages.

A second example of how some of the complexities of natural selection can be analyzed in man issues from a recent study on Huntington's chorea, another dominantly inherited trait (T. Reed and Chandler [40], T. Reed and Neel [42]). Stimulated by a report of the high fertility of individuals with Huntington's chorea in a particular kindred (S. Reed and Palm [39]), several years ago we undertook a study embracing all the families in the state of Michigan in which the disease has ever been recorded as occurring. For all individuals heterozygous for the gene responsible for this disease who met certain necessary restrictions and who had completed reproduction, the relative fitness, defined as the ratio of the mean number of children born to these heterozygotes to the corresponding mean of their normal sibs, was found to be 1.03 ± 0.11, i.e., not different from unity.[1] However, a significant difference was observed between males and females, the value for male heterozygotes being $.82 \pm .11$ and for females $1.25 \pm .14$, although from the clinical standpoint the disease pursues essentially the same course in the two sexes.

[1] The reference to heterozygotes rather than choreics is necessitated by the fact that, in all calculations, allowance must be made for a small amount of non-penetrance.

As in the case of neurofibromatosis, there is evidence that this difference may be due to both a lower marriage rate on the part of male gene carriers and a lower rate of reproduction after marriage. Possibly we see some kind of a general relationship emerging here.

The data were collected in such a fashion as to permit comparison with a properly stratified sample of the female Michigan population as a whole. When this comparison was made, the relative fitness of heterozygotes was estimated to be, not unity, but 0.79 ± 0.12. In other words, there are indications, at about the 5 per cent level of significance, that the relative fitness of both heterozygotes and their normal sibs is lowered, presumably because of psychic factors. The inference is that knowledge of the family background (and possibly other factors) depresses the reproductive performance of both groups. The danger of using sib controls in problems of this nature is apparent.

Individuals with chorea often die or are institutionalized prior to the completion of a normal reproductive span. Several years ago, T. Reed and I proposed the use, in problems of this nature, of a statistic known as "relative reproductive span," defined as weighted relative survival to and through the reproductive period (T. Reed and Neel [41]). Equating date of first institutionalization to death, we find the relative reproductive span for Huntington's chorea to be 0.93. There is the possibility, then, that during their active reproductive lives individuals with Huntington's chorea, especially females, actually reproduce at a slightly greater rate than their normal sibs, but that both groups are below the average reproductive performance of the inhabitants of the state of Michigan. I might say that, among other points, this study has given us a very healthy respect for how extensive a body of data one must have in order to dissect fertility differentials. Finally, infant and childhood mortality is slightly but significantly higher in the children of female choreics than in the children of their normal sibs. We do not have a satisfactory population comparison, but this figure appears less subject to the workings of distorting (psychic) factors which weaken sib comparisons than the one for fertility. We thus have evidence for selection due to mortality differentials at two different stages in the life cycle, evidence for selective factors related to the sex of the affected individual, and, finally, some evidence for a component of selection which may be due to psychic factors. Panse (37) has presented data from Germany on Huntington's chorea which, although not analyzed in this fashion, are susceptible to substantially the same interpretation.

Neither of these cases is by any means completely worked out. The elucidation of many points of interest must await the collection of far more material. Nevertheless, we have here illustrations of how one can begin to unravel the complexities of selection in man. Incidentally, you cannot have failed to note, in view of the findings with respect to the inadequacy of sibling controls in Huntington's chorea, a possible weakness of the study on neurofibromatosis, in which sibling controls were used for fertility estimates, although only the siblings of sporadic cases were utilized, and we have some data to indicate that their fertility is very similar to that of Michigan residents as a whole.

THE INVESTIGATION OF THE GENETIC STRUCTURE OF POPULATIONS

We turn now to consider how we may study the genetic structure of human populations. There's no use pretending we can approach this question with the elegance of the geneticist who works with Drosophila. On the other hand, when we turn to the mammalia, it now begins to appear that we can learn as much from man as from any other species. A high proportion of any wild-trapped collection of mammals either quickly succumbs to disease or fails to breed in captivity. There thus repeatedly arise questions concerning the representativeness of results with many mammal species. Add to this the reluctance of persons with com-

mercial interests in such animals as dogs and cats to reveal the results of such untoward genetic experiments as may develop under their observation, and you have a striking contrast to the situation in man, scattered all over the world in a profusion of different environments, in many of which there is a downright competitive eagerness on the part of some of its members to record their genetic misfortunes.

Three examples of the opportunities which the human species offers for work in this area immediately come to mind. We will be considering a fourth example at some length tomorrow. An important question for any species is the amount of concealed "recessively inherited" variability in the population and the relation of the magnitude of this concealed component to the amount of inbreeding in the population, this latter question having an important bearing on the issue of just how recessive so-called recessive genes really are. The social customs of man vary from the discouragement of consanguineous unions encountered in many European countries and our own, through the tolerance of consanguineous marriage which exists in Japan today, to the very obvious encouragement of consanguinity among the Basuto of southern Africa, where at least one third of a sample of 569 marriages contracted in the Southern Sotho Ward of Basutoland involved consanguinity (Ashton [3]).[2] There is every indication that behind the current practices of these areas lie centuries of tradition, even though for all areas the trend in recent years has probably been toward less consanguinity. No one has as yet been sufficiently enterprising to take real advantage of the Basutoland situation, an oversight which, in view of the current tempo of activity in human genetics, will almost certainly be rectified within the next decade; but we do have some figures on inbreeding effects for Europe (Sutter and Tabah [49, 50]; Böök [5]), and Japan (Yanase [53]; Shiroyama [47]; Ichiba [19]; Morton [29], and Schull [45]). They reveal what to me is surprisingly little difference in the results of first-cousin marriage in the two regions. Obviously, a great deal more in the way of data is needed, collected with due regard for extraneous variables, as well as knowledge of inbreeding coefficients; but the opportunities are clearly there.

A second type of problem in population genetics with respect to which the human species appears to offer unusual possibilities has to do with the significance of congenital defects. Something like 3 per cent of all births result in a child with a rather severe defect apparent before the age of 1 year. It has been customary to regard these unfortunate children as for the most part the accidents resulting from environmental insult or random mutational events. Recently (Neel [34]), I have attempted to pull together a number of scattered leads which collectively point to the possibility that a significant proportion of these children may be the "phenodeviants" resulting from complex, adaptive genetic systems with labile thresholds of expression, of the type discussed in particular by Lerner (25) and Dobzhansky (12). If this hypothesis is correct, children with congenital defect are to some presently unspecifiable extent to be regarded not as nature's mistakes so much as the price human populations pay for certain genetically adaptive systems. The validity of this hypothesis remains to be seen. The point to be made in the present context, however, is that because of man's numbers and subdivisions, together with his increasing concern for recording accurately the outcome of pregnancy, out of the world-wide vital statistics machinery can issue—should issue, with a few improvements—data bearing on the validity of this hypothesis, of a type which for one reason or another would be very difficult to obtain for any other mammal. Lest I appear overenthusiastic, let me recognize that the detailed breeding experiments necessary to nail this hypothesis down cannot be carried

[2] The representativeness of Ashton's population sample seems debatable, but, even if considerable bias exists, the fact of a high degree of consanguinity seems inescapable.

on in man, but require an experimental organism. On the other hand, a great deal pertinent to evaluating the hypothesis can be learned from man. In particular, careful study of the outcome of so-called interracial marriages is called for.

A final illustration of the opportunities offered by man in the field of population genetics stems from recent findings in West Africa as regards the distribution of the abnormal human hemoglobins. Studies on the distribution of the abnormal hemoglobins in West Africa have led to the recognition of two of the most striking gene "clines" or diffusion gradients known for any species. One of these is with respect to the gene responsible for hemoglobin C, with a "high" of approximately 10 per cent in Northern Ghana, and a decreasing frequency as one goes west, north, or east (cf. Neel *et al.* [35]; Lehmann [24]; Allison [2]). It is difficult to escape the impression that the present situation is a most unstable one, with the result that we human geneticists have an opportunity to study the details whereby changes in gene frequency come about, in a way which can be paralleled in only a few other species. Essentially the same situation exists for the gene responsible for hemoglobin S. There is increasing evidence that its introduction to parts of West Africa is, in terms of generations, relatively recent. For instance, Livingstone (26) has just described the following unusual situation in Liberia: a northwest to southeast cline as regards the frequency of this gene, with gene frequencies of approximately 10 per cent in the Mende and Kissi, and essentially zero in the Kru, Krahn, and Webbo. The other limb of this cline, in the Ivory Coast, is under active investigation at present. Inasmuch as the tribes of southeastern Liberia present many paleo-negroid traits which imply a degree of isolation from the surrounding tribes, once again the impression is of a gene on the march. These two situations offer exciting opportunities to come to grips with many questions regarding the details of how one gene replaces another in whole or part in the process of human evolution.

HUMAN BIOCHEMICAL GENETICS

Of all the areas where man seems to have come into the picture as an object of genetic study, biochemical genetics is preeminent. The developments with respect to the human hemoglobins at the moment provide one of the better known documentations of this statement. Human hemoglobin consists of one major and two minor components, the former termed A and the latter A_2 and A_3. Since 1949, no less than eleven inherited variations of the major component of human hemoglobin have been recognized. Because of the rarity of some of these types, as well as their restricted geographical distribution, the task of elucidating the genetic relationships of the genes responsible for these hemoglobins goes slowly, since critical evidence can be derived only from families in which at least two of the responsible genes are segregating simultaneously. The results of studying such families to date can be summarized as follows: The genes responsible for hemoglobins S and C appear to be alleles (Ranney, Larson, and McCormack [38]). There is evidence, unfortunately based on only one family, and thus greatly in need of confirmation, that the gene responsible for hemoglobin G segregates independently from that responsible for hemoglobin S (Schwartz *et al.* [46]). This leads to the postulate that at least two genetic loci contribute to the synthesis of the hemoglobin molecule. Very recently, evidence has appeared suggesting that the genes responsible for hemoglobin S and a new abnormal hemoglobin component, tentatively termed Hopkins-2, are segregating independently (Smith and Torbert [48]). The relationship of the locus responsible for the latter to the locus responsible for hemoglobin G is unknown and, in view of the rarity of both these types, not likely to be determined in the near future. The genetic relationship of the thalassemia abnormality to the hemoglobin S (and, by inference, C) locus is somewhat confused. There is increasing evidence that the traits are not segregating independently (Neel [33], Ceppellini [7]). On the other hand, there are

several families in which the traits have behaved as if due to genes at separate loci (summary in Neel [33]). The findings can be met either by the postulate of linkage or of a genetic heterogeneity of the thalassemia abnormality. The latter possibility would suggest the existence of at least two thalassemia loci, one allelic or closely linked to the S-C locus, the other independent. In summary, then, the fragmentary evidence presently available suggests the existence of several different loci involved in the production of the major component of human hemoglobin. Furthermore, Kunkel *et al.* (23) have recently produced evidence for inherited variations in the minor, A_2 component. The desirability of a speedy clarification of the genetic relationships of the various hemoglobins is underlined by recent developments now to be described concerning their biochemistry.

A considerable amount of work on the nature of the biochemical differences among the various hemoglobins has recently culminated in the very elegant demonstration by Ingram (20) that hemoglobins A, S, and C differ by a single amino acid substitution. The fact that allelic genes control in such a precise manner differences in proteins provides a striking demonstration of the precision of genetic regulation of body structure, a precision long postulated but only now demonstrated. The inference, that the nature and position of each of the 300 amino acids in each of the symmetrical half-molecules comprising human hemoglobin are coded into the chromosomal structures, is obvious. The further inferences concerning that coding system, when we consider the complexity of the human organism, are really rather staggering. Each successive announcement of the structure of an abnormal hemoglobin, as these are worked out, will constitute an important contribution to our understanding of gene action. Our knowledge of the structure of the hemoglobin variants is destined to progress much more rapidly than our knowledge of the genetic relationships of these variants. Here is one of those frustrating situations in human genetics—were man an experimental organism the elucidation of the situation could proceed so much more quickly. The grapevine has it that a number of microbial geneticists are in an urgent quest for a comparable instance of the genetic control of protein structure in a micro-organism, since a precision of genetic analysis never attainable in man can be anticipated. I doubt whether they will ever come up with a protein so conveniently packaged and so available for repeated samplings as hemoglobin.

One final, recent development will serve to illustrate very nicely the ferment in which human biochemical genetics finds itself, and especially the unexpected quarters in which material of great genetic interest may arise. World War II sparked an intense search for new and better antimalarials. Pamaquin (plasmochin) had been recognized in the 1930's as an effective antimalarial (James [21], *et seq.*); systematic studies were now undertaken on this drug, as well as a variety of its analogues (bibliography in "Symposium on Malaria" [51]). While both pamaquin and a number of its analogues such as pentaquin, isopentaquin, and primaquine proved to be highly effective in the prophylaxis of malaria, their use was limited by the frequency of hemolytic reactions encountered following their administration, especially in Negro troops (cf. Hockwald, Arnold, Clayman, and Alving [16]). Since one of these analogues, primaquine, proved to be a highly superior antimalarial, a great deal of interest devolved upon the mechanism of this hemolytic reaction. In a series of classical papers emanating from the Army Malaria Research Unit at the University of Chicago, under the direction of Dr. Alf S. Alving (for bibliography cf. Carson *et al.* [6]), it was shown that the ultimate cause of this susceptibility very probably lies in a loss of activity in the erythrocytes of sensitive persons of the enzyme glucose-6-phosphate dehydrogenase, a loss which in most studies on this system has been measured by a very marked decline in the re-

duced glutathione content of the erythrocytes following incubation with acetyl phenylhydrazine. Such a situation could not long escape the attention of some geneticallly oriented investigator; the recent excellent paper of Childs and his collaborators makes it quite clear that the trait is inherited, the hypothesis most consistent with the data being that the trait (measured by reduced glutathione content rather than enzymatic deficiency) is due to a sex-linked gene of intermediate dominance (Childs *et al.* [8]).

For many years it has been recognized that a small proportion of persons consuming the fava bean, a staple food item in the Mediterranean basin, were subject to a severe hemolytic anemia, a reaction termed "favism." This susceptibility has been noted to exhibit a familial trend (Luisado [27]; Murano [31]; Alcobé [1]; Szeinberg, Sheba, Hirshorn, and Bodonyi [52]). Within the past year it has been shown that individuals who develop favism are apparently deficient in the same enzyme the absence of which is responsible for primaquine sensitivity (Sansone and Segni [43, 44]; Szeinberg *et al.* [52]; Zinkham, Lenhard, and Childs [55]).

Finally, now, to round this story out, there is evidence that the well documented hemolytic reactions occasionally encountered following the administration of sulfanilamide, acetanilide, or nitrofurantoin, or after the accidental ingestion of naphthalene, may all be ascribed to this same enzymatic deficiency (Dern, Beutler, and Alving [11]; Zinkham, and Childs [54]; Kimbro, Sachs, and Torbert [22]). We have here, then, a most instructive example of how the recognition of a genetically controlled enzyme deficiency may unify a diverse and superficially unrelated set of phenomena. How many more such systems remain to be discovered? Not the least fascinating aspect of the system under discussion is that the effect of the enzyme deficiency on individual fitness remains so largely unknown. Childs *et al.* (8) calculate the frequency of the responsible gene in the American Negro as 0.136, from which, because of the known Caucasian admixture, one would infer a gene frequency of approximately 0.20 in the African ancestors of these Negroes. From the known frequency of favism in the Mediterranean basin, comparable gene frequencies probably exist in other ethnic groups. Frequencies of this magnitude for a sex-linked gene in the face of the negative selection imposed by favism clearly imply some selective advantage of the trait, of which nothing is known at present.

A number of recent authors have pointed out the extent to which drug idiosyncrasies may be a means of uncovering previously unsuspected biochemical systems with important genetic implications (Neel and Schull [36]; Haldane [14]; Motulsky [30]). The current flowering of the pharmaceutical industry cannot fail to bring to light large numbers of biochemical differences between individuals. Motulsky (30) has recently enumerated some of the more promising leads for genetic investigation stemming from drug idiosyncrasies. To his list I would like to add the differences among individuals in their ability to acetylate the anti-tuberculous drug isonicotinic acid hydrazide (Hughes, Biehl, Jones, and Schmidt [18]) and the susceptibility of some persons to chlorpromazine jaundice (for the possibility that the latter phenomenon should be interpreted as an allergic reaction, see Hollister [17]). In what other animal species besides man is there such a wealth of biochemical material awaiting investigation?

In closing, just a word about one very practical aspect of research in human genetics—the finances. The geneticist interested in microorganisms or Drosophila can often obtain very significant results with both limited space and budget; the physician interested in medical research can take advantage of many normal medical procedures in the development of his research program; but the human geneticist, with respect to many of the problems that need studying, must seek out, for a variety of special studies, large numbers of persons who would not otherwise be examined. This requires both space and funds. I wonder if

up to this point we have permitted ourselves to think on the appropriate scale. If man's germ plasm is what distinguishes him from the other animals, and if the problems confronting that germ plasm today are as great as we think they are, then surely it is time for an entirely different magnitude of effort. However, this effort can gain momentum no more rapidly than the personnel situation permits, and here there is a critical shortage indeed. In this connection, let me call your attention to the fact that the recently inaugurated Research Training Grant Program of the U.S. Public Health Service specifically recognizes genetics as an acute shortage area. Here is a means for tremendously increasing our training potential. In view of current trends in human biology, there seems no reason to doubt a continuing strong demand for the services of such trainees.

Finally, it remains only to state my firm conviction that, during the last half of the twentieth century, unlike the first half, studies on man will contribute quite as much to our understanding of a wide variety of genetic problems as will studies on any other animal.

REFERENCES

1. ALCOBÉ, S. Estudio genetico del foco de fabismo de Menorca-Ponencia oficial: fabismo y hemolisis alimentaria. IV. Congreso International de Higiene y Medicina Mediterraneas-Barcelona, pp. 53–60, 1953.
2. ALLISON, A. C. The Sickle-cell and Haemoglobin C Genes in Some African Populations. Ann. Human Genet., **21**:67–89, 1956.
3. ASHTON, E. H. The Social Structure of the Southern Sotho Ward. Communications from the School of African Studies, N.S. No. 15, Univ. of Cape Town, pp. 34, 1946.
4. ASHTON, H. The Basuto, pp. xi and 355. London: Oxford University Press, 1952.
5. BÖÖK, J. A. Genetical Investigations in a North Swedish Population. Ann. Human Genet., **21**:191–221, 1957.
6. CARSON, P. E.; FLANAGAN, C. L.; ICKES, C. E.; and ALVING, A. S. Enzymatic Deficiency in Primaquine-sensitive Erythrocytes. Science, **124**:484–85, 1956.
7. CEPPELLINI, R. Studies on the Allelism of the Genes Responsible for Thalassemia and the Sickling Phenomenon (in manuscript).
8. CHILDS, B.; ZINKHAM, W.; BROWNE, E. A.; KIMBRO, E. L.; and TORBERT, J. V. A Genetic Study of a Defect in Glutathione Metabolism of the Erythrocytes. Bull. Johns Hopkins Hosp., **102**:21–37, 1958.
9. CROW, J. F. Some Possibilities for Measuring Selection Intensities in Man. Am. Anthrop., **60**:1–13, 1958.
10. CROWE, F. W.; SCHULL, W. J.; and NEEL, J. V. A Clinical, Pathological, and Genetic Study of Multiple Neurofibromatosis, pp. ix and 181. Springfield: C. C Thomas, 1956.
11. DERN, R. J.; BEUTLER, E.; and ALVING, A. S. The Hemolytic Effect of Primaquine. V. Primaquine Sensitivity as a Manifestation of a Multiple Drug Sensitivity. J. Lab. & Clin. Med., **45**:30–39, 1955.
12. DOBZHANSKY, T. A Review of Some Fundamental Concepts and Problems of Population Genetics. Cold Spring Harbor Symp. Quant. Biol., **20**:1–15, 1955.
13. FISHER, R. A. The Genetical Theory of Natural Selection, pp. xiv and 272. Oxford: The Clarendon Press, 1930.
14. HALDANE, J. B. S. The Biochemistry of Genetics, pp. 144. London: Allen & Unwin, Ltd., 1954.
15. HARRIS, H. An Introduction to Human Biochemical Genetics. Eugenics Laboratory Memoirs, **37**:iv, 96. London: Cambridge Univ. Press, 1953.
16. HOCKWALD, R. S.; ARNOLD, J.; CLAYMAN, C. B.; and ALVING, A. S. Toxicity of Primaquine in Negroes. J.A.M.A., **149**: 1568–70, 1952.
17. HOLLISTER, L. E. Allergy to Chlorpromazine Manifested by Jaundice. Am. J. Med., **23**:870–79, 1957.
18. HUGHES, H. B.; BIEHL, J. P.; JONES, A. P.; and SCHMIDT, L. H. Metabolism of Isoniazid in Man as Related to the Occurrence of Peripheral Neuritis. Am. Rev. Tuberculosis, **70**:266–73, 1954.
19. ICHIBA, M. Study on Children Born as a Result of Consanguineous Marriage. II. On Their Physical and Mental Constitutions. Nihon Ika Daigaku Zasshi, **21**:113–18, 1953.
20. INGRAM, V. M. Gene Mutations in Human Haemoglobin: the Chemical Difference between Normal and Sickle Cell Haemoglobin. Nature, **180**:326–28, 1957.

21. JAMES, S. P. Some General Results of a Study of Induced Malaria in England. Trans. Roy. Soc. Trop. Med. & Hyg., **25**: 477, 1931.
22. KIMBRO, E. L.; SACHS, M. V.; and TORBERT, J. V. Mechanism of the Hemolytic Anemia Induced by Nitrofurantoin (Furadantin). Bull. Johns Hopkins Hosp., **101**: 245–57, 1957.
23. KUNKEL, H.; CEPPELLINI, R.; DUNN, L. C.; and FIRSCHEIM, I. L. A Variant in the Minor Component of Hemoglobin. Fed. Proc. (in press).
24. LEHMANN, H. Distribution of Abnormal Haemoglobins. J. Clin. Path., **9**:180–81, 1956.
25. LERNER, I. M. Genetic Homeostasis, pp. vii and 134. New York: Wiley & Sons, 1954.
26. LIVINGSTONE, F. B. The Distribution of the Sickle Cell Gene in Liberia. Am. J. Human Genet., **10**:33–41, 1958.
27. LUISADO, A. Favism. Medicine, **20**:229–50, 1941.
28. MCKUSICK, V. A. Heritable Disorders of Connective Tissue, pp. 224. St. Louis: C. V. Mosby Co., 1956.
29. MORTON, N. E. Empirical Risks in Consangineous Marriage: Birth Weight, Gestation Time, and Measurements of Infants. Am. J. Human Genet., **10**:344–49, 1958.
30. MOTULSKY, A. G. Drug Reactions, Enzymes, and Biochemical Genetics. J.A.M.A., **165**:835–37, 1957.
31. MURANO, G. Contributo alla conoscenza della predisposizione allergica eredofamilare nel favismo. Pediatria, **59**:479–515, 1951.
32. NEEL, J. V. The Study of Natural Selection in Primitive and Civilized Human Populations. Am. Anthrop., **60**:43–72; Human Biol., **30**:43–72, 1958.
33. ———. Genetic Aspects of Abnormal Hemoglobins. Nat. Res. Council, Conference on Hemoglobin. J. Nat. Acad. Sc. Publ. 557, pp. 253–71, 1958.
34. ———. A study of Major Congenital Defects in Japanese Infants. Am. J. Human Genet., **10**:398–445, 1958.
35. NEEL, J. V.; HIERNAUX, J.; LINHARD, J.; ROBINSON, A. R.; ZUELZER, W. W.; and LIVINGSTONE, F. B. Data on the Occurrence of Hemoglobin C and Other Abnormal Hemoglobins in Some African Populations. Am. J. Human Genet., **8**:138–50, 1956.
36. NEEL, J. V., and SCHULL, W. J. Human Heredity, pp. 361. Chicago: University of Chicago Press, 1954.
37. PANSE, F. Die Erbchorea, eine klinische-genetische Studie. Leipzig: Georg Thieme, 1942.
38. RANNEY, H. M.; LARSON, D. L.; and MCCORMACK, G. H., JR. Some Clinical, Biochemical, and Genetic Observations on Hemoglobin C. J. Clin. Investigation, **32**: 1277–84, 1953.
39. REED, S. C., and PALM, J. D. Social Fitness versus Reproductive Fitness. Science, **113**: 294–96, 1951.
40. REED, T. E., and CHANDLER, J. H. Huntington's Chorea in Michigan. I. Demography and Genetics. Am. J. Human Genet., **10**:201–25, 1958.
41. REED, T. E., and NEEL, J. V. A Genetic Study of Multiple Polyposis of the Colon (with an Appendix Deriving a Method of Estimating Relative Fitness). Am. J. Human Genet., **7**:236–63, 1955.
42. ———. Huntington's Chorea in Michigan. II. Selection and Mutation. *Ibid.* (in press).
43. SANSONE, G., and SEGNI, G. L'instabilita del glutatione ematico (GSH) nel favismo. Utilizzazione di un test selettivo. Introduzione al problemo genetico. Boll. d. Soc. Ital. di Biol. Sper., **33**:1057–60, 1957.
44. ———. Sensitivity to Broad Beans. Lancet, **2**:295, 1957.
45. SCHULL, W. J. Empirical Risks in Consanguineous Marriages: Sex Ratio, Malformation, and Viability. Am. J. Human Genet., **10**:294–343, 1958.
46. SCHWARTZ, H. C.; SPAET, T. H.; ZUELZER, W. W.; NEEL, J. V.; ROBINSON, A. R.; and KAUFMAN, S. F. Combinations of Hemoglobin G, Hemoglobin S, and Thalassemia Occurring in One Family. Blood, **12**:238–50, 1957.
47. SHIROYAMA, E. On the Relation of Growth of Newborn Infants, Sucklings, and Children to Consanguineous Marriage. (Kumamoto Daigaku) Taishitsu Igaku Kenkyūsho Hōkoku, **3**:462–64, 1953.
48. SMITH, E. W., and TORBERT, J. V. Study of Two Abnormal Hemoglobins with Evidence for a New Genetic Locus for Hemoglobin Formation. Bull. Johns Hopkins Hosp., **102**:38–45, 1958.
49. SUTTER, J., and TABAH, L. Effets de la

consanguinité de l'éndogemie. Population, **7**:249–66, 1952.

50. SUTTER, J., and TABAH, L. Structure de la mortalité dans les familles consanguines. *Ibid.*, **8**:511–26, 1953.
51. SYMPOSIUM ON MALARIA. J. Clin. Invest., **27** (Suppl.): 1–150, 1948.
52. SZEINBERG, A.; SHEBA, C.; HIRSHORN, N.; and BODONYI, E. Studies on Erythrocytes in Cases with Past History of Favism and Drug-induced Acute Hemolytic Anemia. Blood, **12**:603–13, 1957.
53. YANASE, T. Heredo-clinical Investigation Concerning the Highly Inbred Villages in Western Japan, Especially Shiba-mura and Miyazaki-ken. Igaku Kenkyu, **21**:183–209, 1951.
54. ZINKHAM, W. H., and CHILDS, B. The Effect of Vitamin K and Naphthalene Metabolites on Glutathione Metabolism of Erythrocytes from Normal Newborns and Patients with Naphthalene Hemolytic Anemia. Am. J. Dis. Child., **94**:420, 1957.
55. ZINKHAM, W. H.; LENHARD, R. E.; and CHILDS, B. A Deficiency of Glucose-6-Phosphate Dehydrogenase Activity in Red Cells from Patients with Favism. Bull. Johns Hopkins Hosp., **102**:169–75, 1958.

The Chromosomes of Man

CURT STERN
Department of Zoology, University of California, Berkeley, Calif.

Human chromosomes were first clearly pictured by Arnold in 1879 (Fig. 1*A*). His drawings from tumor cells show interesting details, including what obviously were multipolar spindles; but they are not suitable for even a rough estimate of the number and types of chromosomes. Flemming, in 1881/82 described and pictured mitoses from normal human tissue. Some twenty large and small chromosomes—V, J, and rod-shaped—are recognizable in a division figure of a corneal cell (Fig. 1*B*). In 1898, Flemming devoted a special note to the topic of the chromosome number in Man (12). He referred to his earlier drawings and stated that they intentionally included only the clearer structures seen. With improved optics Flemming restudied his single remaining slide and concluded, with cautious hesitation, that somatic human cells have certainly more than 22 and probably less than 28 chromosomes and that 24 seemed to be the true number. A succession of later investigators offered different opinions. Estimates or counts in number of chromosomes ranged from 8 to 40 and more with an inclination toward believing 24 to be the correct number.

It was not until the early 1920's that an agreement was even approached. In 1912, on the basis of a thorough study based on superior technique, Winiwarter reported 47 elements in the testes of human males and 48 in the ovaries of females. He concluded that Man belongs to the XX-XO type of sex determination and that the 47 chromosomes consisted of 23 pairs of autosomes and an unpaired X chromosome. Eleven years later Painter (1923) came to a similar view except for one point. He too counted 23 pairs of autosomes and an X chromosome. In addition, however, he saw a small partner to the X, a Y chromosome. Thus, according to Painter, the male, like the female, had 48 chromosomes. Other students (e.g., Minouchi and Ohta [35]) supported the conclusion that Man has 48 chromosomes, although Winiwarter and Oguma (cf. 61) on the basis of their very clearest figures kept on insisting that 47 is the number characteristic for the male. A few investigators reported more divergent numbers, but most of these accounts had obvious weaknesses. They did not change the opinion of the majority that the number of chromosomes in Man had been well established as being 48.

There are more important things to know about Man than the exact number of his chromatic elements. Chromosomes are assemblages of genes. Whether the genes are primarily discrete macromolecules or at least in part integrated into superunits within the chromosomes, evidence in several organisms clearly shows that growth and development can proceed normally on the basis of a multitude of chromosomal rearrangements within a species. There are many ways in which the genes may be packaged, and it would seem of minor consequence whether these packages number 30 or 40 or 50. Nevertheless, Man's chromosome number is at least as significant a biological constant as the number of his teeth, his fingers, or his neck vertebrae. Moreover, it is necessary to know his normal chromosome number if one wishes to determine whether abnormal numbers occur and whether causal relations exist between ab-

normal chromosomal content and abnormal function of cells and tissues.

It was perhaps from the latter angle that the question of the normal number of human chromosomes was reopened again. Following reports by others of variable numbers in different cells of several tissues and, particularly, in cancer cells, the erstwhile botanical cytologists, Tjio and Levan (54), prepared cultures of normal lung fibroblasts from four therapeutically aborted Swedish embryos. Within a few days after the initial explantation they prepared slides with numerous mitotic figures accumulated as the result of colchicine treatment. Tjio and Levan "were surprised to find that the chromosome number 46 predominated in the tissue cultures from all four embryos." Disregarding lower numbers of chromosomes in cells that seemed damaged, they found only four doubtful cases of 47 or 48 chromosomes. No less than 261 cells gave counts of exactly 46 chromosomes. Photomicrographs of two cells in metaphase which accompanied the account are the clearest pictures of the diploid human chromosome assembly yet presented (Fig. 2). While there was the possibility, invocable *ad hoc*, that lung tissue might contain fewer chromosomes than other tissues (particularly the ovaries and testes), Tjio and Levan concluded that it "would be the most natural explanation of (their) observations . . . to generalize (their) . . . present findings into (the) statement that the chromosome number of man is $2n = 46$." Thus, in 1956, 44 years after Winiwarter, and 33 years after Painter had "solved" the problem of the human chromosome number, the question was again raised.

It must seem strange to a non-cytologist and even to cytologists used to the chromosomes of grasshoppers, lilies, and indeed most multicellular and some unicellular organisms, that the normal number of human chromosomes became a matter of inquiry again as late as 1956. All of the means of settling this apparently simple question would have seemed to have been available for at least 60 years. No electron microscope was needed, no photospectroscopy, only some well fixed and stained preparations to be observed under the ordinary light microscope. It must be admitted that this, indeed, was the basically simple situation—but there are attenuating circumstances for the delay in arriving at a final answer. The chromosomes of warm-blooded animals are relatively numerous and crowded into a small nuclear volume. After the use of standard fixation technique they not only tend to lie on top of one another in the optical axis of the microscope but often to clump together (Fig. 7*A*). Even an experienced observer will frequently be unable to decide at many points whether he has one single bent chromosome before his eyes or two straight ones. Then, too, he would have to decide whether two well stained bodies, separated by a light area, are two separate whole chromosomes or only the two arms of a single one whose connecting kinetochore (centromere) had taken up the dye so weakly as to be indistinguishable from the background.

The way out of such ambiguities has been resolved by the use of techniques, partly new and partly old, but wrongly neglected. The *new* techniques involved the use of hypotonic solutions and colchicine pretreatment, resulting in a spreading apart and contraction of the chromosomes (Hughes [20], Hsu [17], Makino and Nishimura [32], Tjio and Levan [59], and Kodani [24]). This reduces the occurrence of optical overlaps of separate chromosomes (Fig. 7*B*). The *old* technique, the revival of which became important, was that of using unsectioned material. Whole cells from small pieces of tissue gently flattened or squashed, or from tissue cultures directly grown on cover glasses, gave better assurance than microtome-sectioned material that no element is lost or no element from another cell mistakenly included in a count. Moreover, in sectioned material the knife may have cut through a chromosome so that consecutive sections may have to be used to reconstruct the whole.

The challenge of Tjio and Levan's discovery of 46 chromosomes in human lung

tissue was soon met in England by Ford and Hamerton (1956) who analyzed the spermatogonia and spermatocytes of testicular biopsy specimens from three adult men. In all three 46 was the spermatogonial number, and 23 pairs were demonstrated most convincingly in the great majority of first spermatocytes (Fig. 4).

Had earlier investigators been mistaken in regarding 48 or at least 47 chromosomes as the correct number? Undoubtedly, error often breeds error. The number of 48, once apparently established, would easily acquire "official" status, and an investigator who would see 46 chromosomes in his slide might well be inclined either to reinterpret the figure as actually possessing 48, or to lay it aside as indecisive. Not even preparations made with the new techniques were immune from variations in interpretations. What seemed to be impressive microphotographs of cells with $2n = 48$ (Hsu [17]) are now held to be compatible with counts of 46 (Hsu [18]).

The power of the number 48 may also have taken hold of Painter (40) when he studied another primate, *Macaca mulatta* (*Rhesus macacus*). Having recognized 48 chromosomes in Man, Painter likewise saw 48 chromosomes in the monkey. Years later, Shiwago (48) in Russia, who could find only 42 chromosomes in the same species, attributed the divergence of results to possible strain differences. After almost 2 decades Darlington and Hague (9) and Chu and Giles (7), with modern techniques and tissue cultures, agree with Shiwago in the count of 42 chromosomes, and so do Rothfels and Siminovitch (1958) and Ford (unpublished). While the possibility of intraspecific variability in chromosome number remains, it appears at present that the old count of 48 for the rhesus monkey was off by six. It is only fair to add that Painter (39) also described the chromosomes of the "Brown Cebus" monkey (a species not identified more specifically) and felt free to report a number different from 48, namely, 54 chromosomes. The newest and at present the only acceptable determinations of chromosome numbers in primates other than Man, according to Darlington and Hague, Chu and Giles, and Bender and Mettler (1958) are 34 (2 species), 42 (6 species), 44 (1 species), 46 (1 species), 50 (1 species), 54 (3 species), 60 (2 species) and 66 (2 species).

Even while Ford and Hamerton (13) were presenting their findings of 46 chromosomes in the testes of Man at the first International Congress of Human Genetics in Copenhagen in August, 1956, Kodani was making ready to describe to the International Genetics Symposia in Tokyo "The caryotype of Man with the diploid chromosome number of 48" (22). Was there then new unresolved divergence of opinion? Soon after, Kodani (23) announced that he had evidence for "three diploid chromosome numbers of Man." Among Japanese, whose testes formed his material, he found some individuals in whom each clearly analyzable cell had 46 chromosomes (unpaired in spermatogonia and mostly paired in spermatocytes), other individuals in whom 48 was the constant number (Fig. 5), and one man whose testicular cells possessed 47 chromosomes. A new and unselected sample of fifteen Japanese (24) consisted of five men with 48, one with 47, and nine with 46 chromosomes. And, for the first time since Tjio and Levan's publication, a white male with 48 chromosomes is also reported by Kodani. Among eight testes from whites in Iowa one had 48 and seven 46 chromosomes.

At present, Kodani is the only cytologist who, since 1956, has found more than 46 chromosomes in normal tissues. Other investigators have encountered uniformly only individuals with 46 chromosomes as seen by Tjio and Levan. Altogether, omitting Kodani's data at the time of writing this review (February, 1958) the published reports giving a count of 46 chromosomes are based on nearly 60 individuals (Tjio and Levan [54], Ford and Hamerton [13], Bender [5], as well as unpublished findings of their own or others kindly communicated to me by Levan and Ford). Nevertheless,

not only Kodani's publications but also limited inspection of his slides at present incline me to accept his claims for the existence of polymorphism in the chromosome number of Man. Yet, more than a single interpretation can be applied even to clear figures, and the possibility of misinterpretation cannot be denied. In order to base one's judgment on general agreement one must ultimately wait and see whether the numbers 47 and 48 will also be reported by others than Kodani.

The nature of the chromosomal polymorphism.—Granted that different men have either 46, 47, or 48 chromosomes, what constitutes the intrinsic differences? Within various animal and plant species, naturally found differences in chromosome number are of two main types. One type consists in a differential distribution of constant chromosomal material. Differential distribution most frequently means that one two-armed chromosome found in some individuals may be represented by two essentially one-armed chromosomes in others. Recent well worked-out examples of this phenomenon which may affect one or more two-armed elements come, among others, from wild populations of grasshoppers (White [58]) and snails (Staiger [51]). Two mammalian species have also been found to contain individuals with widely divergent numbers of chromosomes. Specimens of the rodent *Gerbillus pyramidum* had 40, 52, and 66 chromosomes, but the total number of arms was nearly identical in all individuals, varying only from 74 to 78. It should be added that these different numbers characterize specimens, indistinguishable otherwise, but from different regions of Algeria and Israel. In addition, intrapopulational chromosomal polymorphism in the same species has also been noted in one locality, with 62 and 66 chromosomes, but the same number of chromosome arms was present in all animals (Wahrman and Zohavi [46]). The second mammalian species with chromosomal polymorphism is the common shrew, *Sorex araneus*. About fifty specimens invariably possessed 36 autosomal arms, but these were "assembled" in from 22 to 27 separate chromosomes (Sharman [47], Ford, Hamerton, and Sharman [17]).

Chromosomal polymorphism in Man does not follow the "Robertsonian principle" of a constant number of arms distributed variously among two-armed or one-armed chromosomes. According to Kodani (24), it belongs to a second type of phenomenon which accounts for differences in chromosome numbers. This is the possession by some individuals of chromosome material which is absent in others. "Supernumerary" chromosomes, which were first studied early in this century in plant bugs by E. B. Wilson, are now known to occur in many other insects, flatworms, and various plants, including maize and rye. In Man, Kodani finds that 46 chromosomes, recognizable by their sizes and shapes, are common to all individuals and that individuals with 47 and 48 chromosomes have, in addition to the 46 common elements, one or two extra chromosomes. The supernumerary chromosome is one of the smallest in the complement. It appears similar to the Y chromosome. In the spermatocytes of 47-chromosome individuals it remains unpaired, and in spermatocytes of 48-chromosome individuals the two supernumeraries usually pair. No multiple associations have ever been seen in a metaphase stage. This may be due either to lack of homology between the supernumerary chromosomes and any of the others, or to their shortness, which is correlated with the presence of usually only one chiasma or other type of junction per pair (see below).

As long as the behavior and possible multiple associations of the supernumerary chromosomes in the meiotic prophase are unknown, the origin and homology of this element will remain obscure. Its designation as a supernumerary chromosome implies that it is not an essential part of the human karyotype. The fundamental diploid constitution of Man may thus be said to consist of 46 chromosomes.

It is too early to discuss in detail the relative frequencies of individuals with and

without supernumerary chromosomes. The present limited data indicate that the 46 chromosome type is the most frequent and that, perhaps, the frequencies of 47 and 48 chromosome types are higher in Japanese than in European and American whites. Undoubtedly, the next few years will add considerable information to these topics, from various parts of the world.

One particular aspect already deserves a brief comment. It refers to the apparent paucity of 47 chromosome individuals. With random mating one would expect this group to be more frequent than the rarer of the two other chromosomal types. In reality, only a single individual with 47 chromosomes has yet been found in unselected material. The meaning of this deviation from expectation remains to be seen. It may be parallel to Müntzing's (37) finding on the supernumerary chromosomes of the grass, *Poa alpina*. In the pollen mother cells of different specimens the number of the supernumerary chromosomes ranged from two to eight, with even numbers more frequent than odd ones. As a rule, cells in the root tips do not possess any supernumerary chromosomes. Müntzing tentatively assumes that supernumerary chromosomes in early development have a tendency for nondisjunction. Accordingly, sister chromosomes move together to the same pole, one cell receiving the doubled number and the other none. It is further assumed that nondisjunction is directed in such a way that the future root cells are those which do not receive the supernumerary chromosomes at all, while the future germ cells get the doubled numbers. It is not impossible that such a mechanism may also be active in Man and that the finding of 46 chromosomes in somatic tissues is not an accurate index of the germinal situation. Divergence in chromosome number between somatic and germinal tissues is of regular occurrence in certain groups of insects. Here supernumerary chromosomes are regularly present in the germline, in low numbers or as high as about 80. In early stages of embryogeny they are eliminated from somatic cells so that extreme differences in chromosome number may be found between germ cells and any other type of cell (White [57], Beermann [4]). Whether a similar phenomenon exists in man will have to be resolved by study of somatic and germinal cells from the same individuals.

The karyotype.—Number alone is, of course, one of the least informative aspects of the chromosomes of a species. More specific information is provided by the karyotype, which includes not only numbers but also size, shape, and other individual characteristics of the chromosomes. These attributes are best seen in the more elongate mitotic chromosomes, but the pairs of more contracted meiotic chromosomes can also be used. Drawings and diagrams of the human karyotype prepared by several workers are reproduced in Chart 1. There is a considerable amount of general agreement as to the relative sizes of whole chromosomes and of their arms, but details vary. The longest chromosome is approximately five times the length of the shortest. Tjio and Levan distinguish 10 M chromosomes (median-submedian kinetochore; index, long arm: short arm, 1–1.9), 10 S chromosomes (subterminal kinetochore; index, 2–4.9), and 3 T chromosomes (nearly terminal kinetochore; index, 5 or more), while measurements of Hsu's and Kodani's drawings yield respectively: M, 13 and 16; S, 8 and 11; and T, 1 and 1. Kodani's karyotype includes the supernumerary chromosome, and so does perhaps Hsu's. Also, Kodani's diagram is based on meiotic first metaphase chromosomes instead of mitotic chromosomes and may for this reason give different proportions than those found by the other authors. At least in part the differences probably are due to different treatment of cells. Tjio and Levan emphasize that there is an element of arbitrariness involved in the distinction of M, S, and T chromosomes. Certainly, the present status of our knowledge of the human karyotype is not yet satisfactory.

Details concerning the structure of human chromosomes beyond size and form

have been derived from meiotic stages. The staining behavior of a short region in two autosomes (E and I in Kodani's karyotype diagram) and of all or nearly all of one arm of the X chromosome differs from that of all other chromosomal regions and fits the general characteristic of heterochromatin. Two chromosomes each bear a nucleolus in a specific region. One of these is chromosome L, studied first by Schultz and St. Lawrence (46). The other is a longer chromosome, the identity of which has not been established, since its nucleolus disappears before metaphase when the chromosomes can be defined for purposes of the karyotype diagram.

The pachytene stage of the meiotic prophase has lent itself to further study of chromosomal structure. The L chromosome associated with the larger of the two nucleoli has been described as having 22 chromomeres of various sizes and specific linear arrangement (Schultz and St. Lawrence [46], 1949, Fig. 6 A–B, Kodani [23]). General agreement with these findings has also been expressed by Yerganian (62), who employed a unique technique for the analysis of pachytene chromosomes (Fig. 6*c*). By subjecting fixed testicular tissue to the action of a Waring Blendor he was able to disrupt the cells

CHART 1.—The karyotype of man

I. From a spermatogonium; 46 autosomes plus X and Y (Painter [38]).
II. From a spermatogonium; 46 autosomes plus X (Winiwarter, after Painter [38]).
III. From a spermatogonium; 46 autosomes plus X and Y (Shiwago and Andres [49]).
IV. From a lung cell in tissue culture; treated with colchicine; 46 chromosomes arranged according to their length, in three groups characterized by medium-submedian, subterminal, and nearly terminal position of the kinetochore (Tjio and Levan [54]).
V. Diagrams for 23 autosomes plus X and Y from tissue culture of embryonic spleen (Hsu [17]).
VI. Diagrams for 23 autosomes plus X and Y from first spermatocyte divisions. The thin section in chromosomes E, I, and X signifies heterochromatin. Chromosome L bears a nucleolus (redrawn after Kodani [22]).

and by successive centrifugation of the suspended components of the disassociated tissues to obtain isolated paired pachytene chromosomes. Nine different types of such bivalents have been mapped in some detail (Fig. 6 *D–F*). They are characterized by their distinct forms, specific sequences of larger and smaller chromomeres or knobs, as well as other features. Such cytological mapping of chromosomes is likely to make use of artifacts induced by the treatment, but the relative constancy of their main features suggests that the artifacts are vitally preformed, depending on some type of differentiation of the chromosomes which is present in the living stage. It will, however, be necessary to complement these studies by the difficult analysis of chromosomes of whole nuclei in order to make more reliable the interpretation of isolated chromosomes and chromosome fragments.

The bivalent chromosomes of first meiotic metaphases are held together by what appear to be typical chiasmata (except for the XY pair which will be discussed separately). Ford and Hamerton have counted the chiasmata present at metaphase in a total of 23 cells and obtained a mean of about 56 chiasmata for the 23 chromosome pairs. The largest bivalent frequently has five chiasmata, and others may have as many as four. The smaller chromosomes often possess only a single chiasma per bivalent.

There has been a report that finer differences in chromosomal constitution may be observed among different men. Andres and Navashin (1) measured the relative lengths of six pairs of spermatogonial chromosomes in their own slides from two Russians and in a slide from one Japanese, loaned to them by Minouchi. They found no differences in three pairs but from 20 to 50 per cent greater lengths in the three largest chromosomes of the Japanese individual. Such differences perhaps depend on variations in degree of coiling of the chromosomes. Chromosome lengths are known to be gene-controlled in some plants. It is, however, not impossible that the differences observed by Andres and Navashin are consequences of non-genetic differences in the tissues they studied. Evans and Swezy (1928) measured the lengths of all chromosomes in ten mesenchyme cells from each of one male and one female, embryo and adult individual. They found chromosomes in the males to be considerably longer than those in the females, particularly so for the largest chromosomes. A similar sex relation was obtained for the chromosomes of two rat embryos, one male, one female. No other investigators have attempted to study this relation which at present can only be cited without further comment. (It can only be mentioned here that Shiwago [48] compared the morphology of the ten largest chromosomes of man with those of the rhesus monkey. There were some clear differences pair by pair, but on the whole a considerable degree of similarity. More extensive differences have been reported by Darlington and Hague [9].)

Other variations in chromosome characters have been suggested by findings of bridge-like connections between separating anaphase groups and of chromosome fragments in first divisions of spermatocytes of certain individuals (Koller [25], Slifer and Beams [50]; Fig. 3). These phenomena are typical for inversion heterozygotes, i.e., individuals in which the sequence of loci in one or two homologous chromosomes is inverted (e.g., 1-5-4-3-2-6-7, in relation to the other, 1-2-3-4-5-6-7). Apparent bridges and fragments can also be produced by adhesions of chromosome ends and laggard whole smaller chromosomes, and it is not known what the true cause of the unusual patterns of anaphases has been (Schultz and St. Lawrence). It is unlikely that inversions will not be found in Man, since they occur in many other organisms.

Kodani (24) has observed a single spermatocyte among fifteen of a 48-chromosome testes in which, in addition to the 24 pairs, there was a univalent extra chromosome, larger than the supernumerary. Probably, this extra chromosome owed its presence to nondisjunction. There exists a well docu-

mented case in the literature of a woman belonging to blood group AB, married to an O man, who bore a very deformed O child. It has been suggested that this child possibly came from an egg which, in consequence of nondisjunction, had received neither of the homologous chromosomes carrying the I^A or I^B blood group alleles (Levine, after Wiener [54, p. 185]). If this is indeed the explanation, then the presence of the abnormalities in the child could be hypothetically attributed to its possessing only a single chromosome of the relevant pair and this derived from the father. Levine's explanation may well be valid, but apart from mutation the recent discovery of a suppressor gene for blood group substance B (29) provides an alternative way of accounting for an apparent O child from an AB parent, although it would leave unexplained the presence of congenital malformations.

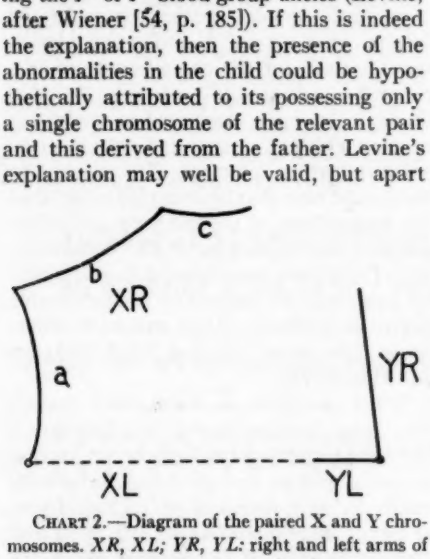


CHART 2.—Diagram of the paired X and Y chromosomes. *XR, XL; YR, YL:* right and left arms of the two chromosomes. *a, b, c,* the three segments of *XR*. The broken line for *XL* signifies heterochromatin (Kodani [22]).

The sex-chromosomes and sex-chromatin.—The nature and behavior of the sex chromosomes in Man have been a subject of much discussion. As stated earlier, Winiwarter, in 1912, on the basis of his counts of 47 chromosomes in testicular and 48 in ovarian tissues concluded that the male has a single unpaired sex chromosome, the X, and that the female has two X chromosomes. Painter (38, 39) observed both a relatively large X chromosome in the male and a small Y. His findings were upheld by various other cytologists, but Winiwarter supported by Oguma strongly maintained his original position (cf. 61). It is possible that their interpretation of cells as containing an XO type of sex-chromosomal constitution was partly based on 47-chromosome material in which the odd chromosome was a supernumerary one. However, the cytological methods available in earlier years made definitive conclusions difficult, and other cytologists (e.g., Minouchi and Ohta [35]) interpreted published figures with supposedly 47 chromosomes as actually showing 48 chromosomes.

There seems to be no doubt anymore that the human male, like most other mammals, possesses both an X and a Y chromosome (see the critical discussion by Matthey [33] and, among more recent authors, Sachs [43], Ford and Hamerton [13], and Kodani [23]). The two sex chromosomes form an unusually shaped bivalent in the first spermatocyte which makes it easily recognizable (note the remarkable similarity of this pair in some of the cells studied by Ford and Hamerton and by Kodani, Figs. 4 and 5). The X chromosome is of medium size, and the Y chromosome is one of the smallest of the complement. According to Kodani (23), the X chromosome consists of two arms, a long one in which three euchromatic sections are distinguishable by their being somewhat abruptly bent away from one another and a somewhat shorter heterochromatic arm which possibly terminates in a small euchromatic section (Chart 2). The Y chromosome consists of two euchromatic arms whose length are in the proportion of about 2:1. In bivalents the shorter arm is terminally joined to the tip of the short arm of the X chromosome. The nature of the meiotic association of the X and Y chromosomes has been the subject of much and sometimes sharp controversy. Matthey (34) has given a detailed analysis of the findings of himself and of others on the behavior of both human and non-human mammalian sex chromosomes. It now seems

likely that no true chiasmata are formed between the X and Y.

In 1952, Graham and Barr noted "a sex difference in the morphology of metabolic nuclei in somatic cells of the cat." The difference consists in the presence of a small, deeply stainable body in most nuclei of certain ganglion cells from females and the absence of this "sex-chromatin" in the majority of corresponding nuclei of males. Similar differences in sex-chromatin were soon found between human females and males in various tissues. The "Barr test" for sex-chromatin has become a powerful tool in the cytological analysis of human sex-deviants.

The discovery of sex-chromatin in mammalian nuclei was made without awareness of older work, but the phenomenon of persistent staining in non-dividing nuclei of parts of the sex-chromosomes which seems to be the basis of the sex-chromatin had long been analyzed in insect tissues. Sachs and Danon (45) have given particular attention to the cytological interpretation of what these authors call human "chromocenters" and to variations in their appearances in different tissues. In young spinous cells of the human skin they often find two separate, rather large, and equal-sized chromocenters in females but only a single such chromocenter plus a very small one in males. These they regard as of X and Y chromosomal origin, respectively. In a certain type of genetic intersexes, Danon and Sachs (8) find "that there is an excess of nuclei with three chromocenters in all their [i.e., the intersexes] skin cells." The authors do not make it clear whether they regard this as evidence of XXX or of XXY constitution, and one must look forward to further studies which may be of great importance for our understanding of the chromosomal determination of sex in Man.

The DNA content of human chromosomes.—As is well known, within a given species the amount of deoxyribonucleic acid (DNA) in the nuclei of diploid cells is usually constant. It is double that in the haploid gametes. In Man, Mirsky and Ris (36) found about 6×10^{-9} mg. of DNA in diploid nuclei.

Since the DNA is localized in the chromosomes and in all likelihood represents the material responsible for the transmission of genetic specificity, its quantity would seem to be a fundamental property of a cell. It is, therefore, at first surprising to learn that the DNA content of the sperm of a sample of 21 infertile men was found to be approximately one half of that of fertile men (26). No direct chromosome studies were made. DNA determinations of primary and secondary spermatocyte nuclei of the infertile males gave amounts four times and two times, respectively, greater than their sperm cells. This suggests that meiosis had proceeded as in fertile men, reducing the chromosomes from the four chromatid stages of the bivalents in the late meiotic prophase of the two chromatid stage of diakinesis and then to the single chromatid stage of the spermatid. If fertile men are characterized by DNA amounts in first and second spermatocytes and sperm of 4, 2, and 1 arbitrary units, the infertile on the average had 2, 1, and $\frac{1}{2}$ units, respectively. One may wonder whether each chromosome in the germ line of the sterile individuals is endowed with only one half of the DNA of that of chromosomes of fertile men. It would seem that chromosomes are composed of numerous identical chromosomal subunits. The cable-like multi-strand "polytene" chromosomes made famous by the salivary glands of many diptera would appear to be possible but not unique models for the composition of more ordinary chromosomes. The chromosomes of sterile men may then have fewer replicated subunits than those of fertile men.

A comparison between the DNA content of the nuclei of the mouse, the rat, and a variety of other mammals with that of Man gives very similar values for all of them (Mirsky and Ris [36], Vendrely [55]). The DNA content of Drosophila nuclei is only about one thirtieth of that of human nuclei. Since it seems unlikely that this means that man has 30 times as many

genes as the fly, the interpretation again is rather that the chromosomes of Man are composed of more equivalent subunits than those of the fly.

The chromosomes in somatic tissues and in tissue cultures.—This review cannot attempt a detailed discussion of the important work on chromosome numbers and karyotypes in *in vivo* and *in vitro* cultures of normal and tumorous human cells and on the related problems of constancy or variability of chromosomal numbers and types in somatic tissues (Beatty [3], Hsu [18]). The chromosome content of cultured cells has been shown to undergo numerous changes. It is presumed that, as a consequence of non-disjunction and a variety of aberrant division processes, cells with heteroploid, tetraploid, and higher multiple chromosome numbers originate (cf. Hsu and Moorhead [19]). On the other hand, multipolar mitoses followed by multiple cell divisions will lead to cells with subdiploid constitutions. Superimposed on these processes spontaneous chromosome breakages are observed with surprising frequency. They result in chromosomal reorganizations of various types such as translocations, formation of ring chromosomes, chromosomes with two kinetochores, and acentric chromosome fragments.

Not many years ago the majority of cytogeneticists would have assumed with confidence that most, if not all, changed karyotypes would exist only temporarily, since the normal balance of genes is absent. But this assumption and confidence has proved unjustified. The cells of most if not all known tumor strains have highly aberrant karyotypes, with the majority of strains characterized by cells with one of several hyperdiploid numbers and other strains consisting primarily of hypodiploid, hypotetraploid, or hyper-tetraploid cells (see Levan [27, 28], Ising and Levan [21]). Even strains with an identical or similar number of chromosomes may have very different karyotypes. In one strain specific chromosomes may all be present in equal numbers, while in another some may be present in excess and others under-represented. Or, rearrangements such as translocations may have distributed differently the chromosomal material over equal number of chromosomes. Often, within one strain there exists a variability in chromosome numbers, greater than is accountable by uncertainties in technique.

The main karyotype of each strain is perpetuated from cells which serve as a stemline. Other stemline cells, of different karyotypes, coexist in many strains and may become dominant with changes in the environment. Furthermore, reorganizations of karyotypes may occur so that the very concept of a constant stemline is only a relative one (cf., Makino [30, 31], Hsu [18]). Clearly, in tumor strains, as well as in strains derived from non-tumorous cells, new balances become established which under the conditions of culturing are selectively superior to those represented by the normal karyotype. These discoveries raise anew the question of the karyotype in somatic tissues within the intact individual. Formerly, the findings of abnormal chromosome numbers in somatic tissues, if technically acceptable, could well be thought of as of temporary significance only. Cells with such abnormal numbers were expected to have a short life in terms of either their own survival or that of their descendents. However, if cells in culture can be superior selectively by means of or in spite of abnormal karyotypes, there is less reason to insist that permanent tissues must be characterized by the karyotype found in the germline. The bearing of the cytological findings first made on cultures of mouse and rat tumor cells and now on cultures of human cells seems to go considerably beyond their significance for the cancer problem and to extend to the problems of normal differentiation. The old recognition that an understanding of normal growth and development is necessary for that of tumors finds its counterpart in the new insight into normal differentiation which the study of tumors may ultimately provide.

Chromosomes in human tissue cultures can be subjected to treatment such as radiation or exposure to chemical agents. Treatments of this kind have long been used within the intact body, particularly in con-

nection with the therapy of tumors. *In vitro* cultures—particularly if derived from single cells—make available for controlled experiments human cells and their chromosomes. Puck (42) has obtained survival curves for human cell populations after irradiation with x-rays which he interprets in terms of damage to two randomly distributed loci in the chromosomes. Bender (5) studied directly human chromosomes in tissue cultures after irradiation. In control cultures one or more chromosome fragments were observed in nearly 1 per cent of all cells. After irradiation with as little as 25 and 50 r units the percentages of fragments were 6 and 15, respectively. This high sensitivity of human chromosomes to irradiation is in line with expectation derived from animal experiments, but the data on human material obviously carry the highest weight in the evaluation of the effects of radiation on Man.

Human chromosomes as bearers of genes.—The orderly segregation and recombination of human genes, as observed by their consequences in successive generations and deduced from population-genetic considerations, place them in the chromosomes. In this, Man, of course, does not deviate from all other organisms which possess chromosomes. In detail, our knowledge of the chromosomal localization of genes in Man is still much more limited than that of many of the genetically more explored animals and plants.

At present only one single chromosome can be correlated with known genetic functions. This is the X chromosome, which has been identified since 1911 as the carrier of "sex-linked" (better designated as X-linked) traits (Morgan, Wilson). Many such X-linked genes have been found.

A number of published pedigrees seemed to show hologynic transmission of genes for several generations—from mothers to all of their daughters and none of their sons. These pedigrees had been tenatively interpreted in terms of X-linked genes with the peculiarity, known from strains of Drosophila, that the two X chromosomes of the human mother were assumed to be permanently attached to one another. A review of the best known human case of this kind, as well as the others, has removed much of the evidence for attached X chromosomes in Man (53).

The Y chromosome has been considered as the carrier of genes responsible for holandric transmission—from fathers to all of their sons and none of their daughters. Again, critical review failed to sustain sufficiently the claims of the Y chromosome for being the seat of any known genes (Stern [52], Penrose and Stern [41]). Not even the role of the Y chromosome in sex determination is understood. Nature's introduction of two instead of one variable in the genotypes responsible for the female-male difference, *viz.*, the 2X-1X *plus* the no Y-1Y alternative, has left the matter obscure. Does an XX conceptus develop into a female because it has two X chromosomes, because it has no Y, or because both facts play a role in its sexual determination? Is a man a man because he has only one X chromosome, or because he has a Y, or because of both? Perhaps the study of sex-chromatin in intersexes, after the manner initiated by Sachs and Danon, will bring us the answer soon. (***Addendum added in proof.*** It has now been established, in Man and the mouse, that the Y chromosome determines maleness.)

For many years the X and Y chromosomes have been considered as probably carrying, in homologous segments, allelic genes which cross over, back and forth, from one sex chromosome to the other. The search for partial sex linkage has been fascinating and methodologically fruitful (Haldane [16] and later). But the case for existence of partial sex linkage has become so weak that no fully convincing example remains. Even the earlier cytological evidence for chiasmata between the X and Y chromosomes has been disputed and been declared as completely wanting (Sachs [44], Matthey [34]). Matthey has proclaimed the emancipation of the cytologist from the domination of the geneticist. On his part, the geneticist should be free to continue his probing for partial sex linkage independent of the findings of the cytologist.

Should this type of gene transmission ever be established, its physical basis will also be found.

The supernumerary chromosome of Man may perhaps deserve the negative distinction of being definable as free from specific genes. Its non-essential nature is obvious from its absence in individuals with 46 chromosomes. And the analogy with supernumerary chromosomes in other organisms suggests that it is made up of "non-specific" genic material—whatever this may actually mean.

None of the numerous autosomal genes in Man can be assigned to any particular one of the regular 22 pairs of autosomes. The future discovery of structurally variant homologous autosomes in different individuals and findings of correlations between their transmission and that of specific genes could lead to such assignments. Perhaps, even before this kind of knowledge is derived from work with individuals and populations, correlated cytological and genetic studies of cultured human cells will lead to localizations of genes in definite chromosomes.

Most human genes when followed simultaneously in transmission have been found to recombine independently. The presence of 23 chromosomal pairs makes the probability small of encountering two genes which are linked in the same chromosome. Furthermore, even genes located together in one chromosome but sufficiently far apart may be interchanged so frequently that they are virtually independent. Ford and Hamerton have called attention to the high frequency of chiasmata in Man and derived from it the expectation that crossing over between linked genes may have a very high frequency. This may be true, though to a limited extent only if the chiasmata—regarded as the sites of crossing over—would be found to be more or less restricted to limited regions in many chromosomes.

Obviously, all X-linked genes are located in the same chromosome, but attempts to map the X chromosome have been limited by the rareness in which heterozygosis for two X-linked genes is encountered in the same woman. (Simultaneous heterozygosis for three or more X-linked loci has never been seen yet.) Three 2-point linkages in the X chromosome have now been established, all of them involving the locus for red-green color blindness. One of the recombination values is of the order of 10 per cent, another about 25 per cent. The third is so close to 50 per cent that it is equivalent to free recombination and only recognizable as linkage by the X-linked transmission of the genes involved. The three two-point maps derived from these linkages cannot yet be combined into a unified four-point map.

Indications for autosomal linkages have been obtained in pioneer surveys by several investigators. Unequivocal evidence is still very limited, but three two-point linkages now seem well established with recombination values of between 3 and 10 per cent. They mark specific short stretches of chromosomes, but which chromosomes and which part of the chromosome the stretches represent is completely unknown.

The sum of the map lengths of all autosomes, estimated by Ford and Hamerton on the basis of chiasmatic frequency, is about 2700 crossover units. The sum of the three short stretches actually established is less than 1 per cent of this suspected total. More than 99 per cent of the genetic lengths of the human chromosomes remains to be mapped.

It would, however, be utterly misleading to end this survey of the human chromosomes in a pessimistic vein. We know so much more about the chromosomes of Man today than only a few years ago. We have abandoned misconceptions. We are working with new tools, with new ideas. Certainly, gaps will be filled and insights gained in steady progression.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. Aloha Hannah-Alava for help in the preparation and critical review of the manuscript of this paper.

REFERENCES

1. ANDRES, A. H., and NAVASHIN, M. S. Morphological Analysis of Human Chromosomes. Proc. Maxim Gorky Med. Gen. Res. Inst. (Moscow), **4**:506–24, 1936.
2. ARNOLD, J. Beobachtungen über Kerntheilungen in den Zellen der Geschwülste. Virchow's Arch., **78**:279–301, 1879.
3. BEATTY, R. A. How Many Chromosomes in Mammalian Somatic Cells? Intern. Rev. Cytol., **3**:117–97, 1954.
4. BEERMANN, W., Nuclear Differentiation and Functional Morphology of Chromosomes. Cold Spring Har. Symp. Quant. Biol., **21**: 217–32, 1956.
5. BENDER, M. A. X-ray Induced Chromosome Aberrations in Normal Diploid Human Tissue Cultures. Science, **126**:974–75, 1957.
6. BENDER, M. A., and MITTLER, L. E. Chromosome Studies of Primates. Science, **128**:186–90, 1958.
7. CHU, E. H. Y., and GILES, N. H. A Study of Primate Chromosome Complements. Am. Nat., **91**:273–82, 1957.
8. DANON, M., and SACHS, L. Sex Chromosomes and Human Sexual Development. Lancet, **273**:20–25, 1957.
9. DARLINGTON, C. D., and HAGUE, A. Chromosomes of Monkeys and Men. Nature, **175**:32, 1955.
10. EVANS, H. M., and SWEZY, O. The Chromosomes in Man, Sex and Somatic. Mem. Univ. Calif., **9**:1–64, 1929.
11. FLEMMING, W. Beiträge zur Kenntniss der Zelle und ihrer Lebenserscheinungen. Part III. Archiv. f. Mikroskop. Anat., **20**:1–86, 1882.
12. ———. Ueber die Chromosomenzahl beim Menschen. Anat. Anz., **14**:171–74, 1898.
13. FORD, C. E., and HAMERTON, J. L. The Chromosomes of Man. Nature, **178**:1020–23, 1956.
14. FORD, C. E.; HAMERTON, J. L.; and SHARMAN, G. B. Chromosome Polymorphism in the Common Shrew. Nature, **180**:392–93, 1957.
15. GRAHAM, M. A., and BARR, M. L. A Sex Difference in the Morphology of Metabolic Nuclei in Somatic Cells of the Cat. Anat. Rec., **112**:709–23, 1952.
16. HALDANE, J. B. S. A Search for Incomplete Sex-Linkage in Man. Ann. Eugen., **7**:28–57, 1936.
17. HSU, T. C. Mammalian Chromosomes *in Vitro*. I. The Karyotype of Man. J. Hered., **43**:167–72, 1952.
18. ———. Numerical Variation of Chromosomes in Higher Animals. *Ibid.* (in press).
19. HSU, T. C., and MOORHEAD, P. S. Mammalian Chromosomes *in Vitro*. VII. Heteroploidy in Human Cell Strains. J. Nat. Cancer Inst., **18**:463–71, 1957.
20. HUGHES, A. Some Effects of Abnormal Tonicity on Dividing Cells in Chick Tissue Cultures. Quart. J. Micro. Sc., **93**:207–20, 1952.
21. ISING, U., and LEVAN, A. The Chromosomes of Two Highly Malignant Human Tumours. Acta Pathol. et Microbiol. Scandinav., **40**:13–24, 1957.
22. KODANI, M. The Caryotype of Man with the Diploid Chromosome Number of 48. Proc. Int. Genet. Symp. Cytol. Suppl., pp. 103–7, 1957.
23. ———. Three Diploid Chromosome Numbers of Man. Proc. Nat. Acad. Sc., **43**:285–92, 1957.
24. ———. The Supernumerary Chromosome of Man. Am. J. Human Genetics, **10**:125–40, 1958.
25. KOLLER, P. C. The Genetical and Mechanical Properties of Sex Chromosomes. III. Man. Proc. Roy. Soc. Edinb., **55**:194–216, 1937.
26. LEUCHTENBERGER, C.; WEIR, D. R.; SCHRADER, F.; and LEUCHTENBERGER, R. Decreased Amounts of Desoxyribose Nucleic Acid (DNA) in Male Germ Cells as a Possible Cause of Human Male Infertility. Acta Genetica et Stat. Medica, **6**:272–78, 1956.
27. LEVAN, A. Chromosome Studies on Some Human Tumors and Tissues of Normal Origin, Grown *in Vivo* and *in Vitro* at the Sloan-Kettering Institute. Cancer, **9**:648–63, 1956.
28. ———. Chromosomes in Cancer Tissue. Ann. N.Y. Acad. Sc., **63**:774–89, 1956.
29. LEVINE, P.; ROBINSON, E.; CELANO, M.; BRIGGS, O.; and FALKINBURG, L. Gene Interaction Resulting in Suppression of Blood Group Substance B. Blood, **10**:1100–1108, 1955.
30. MAKINO, S. The Concept of Stemline-Cells as Progenitors of a Neoplastic Population. Proc. Intern. Genetics Symp., 1956 Suppl. Vol. Cytologia: 177–81, 1957.
31. ———. The Chromosome Cytology of the Ascites Tumors of Rats, with Special Refer-

ence to the Concept of the Stemline Cell. *In:* G. H. BOURNE and J. F. DANIELLI (eds.), Intern. Rev. Cyt., **6**:25–84. New York: Academic Press, 1957.

32. MAKINO, S., and NISHIMURA, I. Water-pretreatment Squash Technic. Stain Technol., **27**:1–7, 1952.
33. MATTHEY, R. Les Chromosomes des vertébrés. Lausanne: F. Rouge, 1949.
34. ———. Les bases cytologiques de l'hérédité "relativement" liée au sexe chez les mammifères. Experientia, **13**:341–46, 1957.
35. MINOUCHI, O., and OHTA, T. On the Number of Chromosomes and the Type of Sex Chromosomes in Man. Cytologia, **5**:472–90, 1934.
36. MIRSKY, A. E., and RIS, H. The Desoxyribonucleic Acid Content of Animal Cells and Its Evolutionary Significance. J. Gen. Physiol., **34**:451–62, 1951.
37. MÜNTZING, A. Accessory Chromosomes in *Poa alpina*. Heredity, **2**:49–61, 1948.
38. PAINTER, T. Studies in Mammalian Spermatogenesis. II. The Spermatogenesis of Man. J. Exper. Zool., **37**:291–321, 1923.
39. ———. The Sex Chromosomes of Man. Am. Nat., **58**:506–24, 1924.
40. ———. Studies in Mammalian Spermatogenesis. IV. The Sex Chromosomes of Monkeys. J. Exper. Zool., **39**:433–62, 1924.
41. PENROSE, L. S., and STERN, C. Reconsideration of the Lambert Pedigree (*Ichthyosis hystrix*). Ann. Human. Genetics, **22**: 258–83, 1958.
42. PUCK, T. T. The Genetics of Somatic Mammalian Cells. Adv. Biol. & Med. Phys., **5**:75–101, 1957.
43. SACHS, L. Sex-linkage and the Sex-Chromosomes in Man. Ann. Eugen., **18**:255–61, 1954.
44. ———. The Possibilities of Crossing-over between the Sex Chromosomes of the House Mouse. Genetics, **27**:309–22, 1955.
45. SACHS, L. and DANON, M. The Diagnosis of the Sex Chromosomes in Human Tissues. Genetics, **28**:201–16, 1956.
46. SCHULTZ, J., and ST. LAWRENCE, P. A Cytological Basis for a Map of the Nucleolar Chromosome in Man. J. Hered., **40**:30–38, 1949.
47. SHARMAN, G. B. Chromosomes of the Common Shrew. Nature, **177**:941–42, 1956.
48. SHIWAGO, P. I. Recherches sur le caryotype du *Rhesus macacus*. Bull. Biol. et Med. Exper., **8**:3–13, 1939.
49. SHIWAGO, P. I., and ANDRES, A. H. Die Geschlechtschromosomen in der Spermatogenese des Menschen. Ztschr. Zellforsch, **16**:413–31, 1932.
50. SLIFER, E. H., and BEAMS, H. W. Chromatid Bridges and Fragments in Human Spermatocytes. Am. J. Human Genetics, **1**:79–82, 1949.
51. STAICER, H. Der Chromosomendimorphismus beim Prosobranchier *Purpura lapillus* in Beziehung zur Ökologie der Art. Chromosoma, **6**:419–78, 1954.
52. STERN, C. The Problem of Complete Y-linkage in Man. Am. J. Human Genetics, **9**:147–66, 1957.
53. STERN, C., and WALLS, G. L. The Cunier Pedigree of "Color Blindness." Am. J. Human Genetics, **9**:249–73, 1957.
54. TJIO, J. H., and LEVAN, A. The Chromosome Number of Man. Hereditas, **42**:1–6, 1956.
55. VENDRELY, R. The Deoxyribonucleic Acid Content of the Nucleus. *In:* E. CHARGAFF and J. DAVIDSON (eds.), Nucleic Acids, **2**: 155–80, 1955.
56. WAHRMAN, J., and ZAHAVI, A. Cytological Contributions to the Phylogeny and Classification of the Rodent Genus *Gerbillus*. Nature, **175**:600, 1955.
57. WHITE, M. J. D. Cytological Studies on Gall Midges (Cecidomyidae). Univ. Texas Pub. **5007**:5–80, 1950.
58. ———. Adaptive Chromosomal Polymorphism in an Australian Grasshopper. Evolution, **10**:298–313, 1956.
59. WIENER, A. S. Blood Groups and Transfusion, 3d ed. Springfield, Ill: Charles C Thomas, 1943.
60. WINIWARTER, VON H. Études sur la spermatogenése humaine. I. Cellule de Sertoli. II. Hétérochromosome et mitoses de l'épthélium séminal. Arch. Biol., **27**:91–189, 1912.
61. WINIWARTER, DE H., and OGUMA, K. La Formule chromosomiale humaine. Arch. Biol., **40**:451–553, 1930.
62. YERGANIAN, G. Cytologic Maps of Some Isolated Human Pachytene Chromosomes. Am. J. Human Genetics, **9**:42–54, 1957.

Methodology in Human Genetics*

ARTHUR G. STEINBERG
Department of Biology and Department of Preventive Medicine, Western Reserve University
Cleveland, Ohio

INTRODUCTION

The study of human genetics is complicated by our inability to control matings and by the fact that the length of a generation of the experimental organism is the same as that of the investigator. For these reasons and not because the genetics of man is basically different from that of other organisms, the human geneticist interested in problems other than gene action is required to use statistical methods of analysis, which geneticists working with other organisms need not employ.

Detailed analysis of hereditary patterns in man is limited for technical reasons almost entirely to characters determined by single gene differences. In general, the sequence of questions concerning the genetics of a character appears to be:

1. Is the character hereditary?
2. If so, what is the genetic mechanism?
3. How frequent is the gene in the population?
4. What is its mutation rate?
5. What are its linkage relations?

Another series of problems concerns genes in populations and populations of genes such as:

1. How do gene frequencies vary among populations?
2. How do they vary among groups (isolates) within a population?
3. What is the average mutation rate of a group of genes?
4. What is the average number of mutations carried by man?
5. What is the rate of response of human genes to various mutagens; in particular, to ionizing radiations?

Even if I were competent to do so, time would not permit me to review the methods of analysis used in these several problems. I have decided, therefore, to limit my discussion to some areas which the physician who is not a geneticist is most likely to want to use or to encounter. In the course of my presentation I shall provide references to more detailed discussions for those who wish further information.

David and Snyder (10) (p. 1130) asked, "What are the necessary and sufficient criteria for establishing that genetic constitution is significant in the etiology of a disease?" and answered (p. 1132), "An incidence of cases among the relatives of propositi significantly in excess of the incidence in the general population can be taken as substantial evidence that genetic factors are etiologically involved, *but only if it is shown that environmental factors cannot account for the increased incidence.*" (Italics provided.) David and Snyder are not the only ones to have posed and answered this question, nor were they the first. Indeed, most workers in human genetics have raised this question and answered it in the same way. I cite David and Snyder because of their excellent discussion of the difficulties which may be encountered in establishing this point.

DETERMINATION OF THE PATTERN OF INHERITANCE

If a significant increase in incidence, not due to environmental factors, has been found among the relatives of the probands,

* Supported in part by grants A-1822, A-1831, and H-3708 from the National Institutes of Health.

an attempt may be made to establish the genetic mechanism responsible for this increased incidence. As has already been stated, in practice only mechanisms involving single gene differences can be adequately analyzed in man. These differences may concern genes on the X chromosome or on some one of the autosomes. In either case, the mutant allele may be dominant, incompletely dominant, or recessive. In some instances patterns involving incomplete penetrance, situations in which those having the given genotype do not have a changed phenotype (see Snyder and David [37] for an excellent discussion), may be analyzed.

Autosomal dominant genes.—The simplest case is that of a rare, autosomal dominant gene with complete penetrance; here we expect one half of the parents, one half of the sibs, and one half of the offspring of the probands to be affected.

The expected values will be realized in large samples, provided we are careful to consider how the sample was selected. If the sample was selected via affected parents, 50 per cent of the offspring may be expected to be affected. What proportion may we expect to be affected if the families are selected via affected children? If we fail to take into account the way the families were selected, far more than 50 per cent of the offspring will be found to be affected.

As an example, consider the data reported by a nongeneticist, under the title, "Frequency of retinoblastoma in the progeny of parents who have survived the disease" (37). The data were derived from fifteen families, each with one parent who had survived the disease. There were 30 children in these fifteen families. Twenty-three of the 30 had had retinoblastoma, and only seven were healthy. This ratio is clearly closer to 3:1 than to the 1:1 expected. Fourteen of the fifteen families were ascertained because an affected child had been brought to the physician for treatment. It is clear that no family selected via an affected child can have fewer than one affected child. Hence, families of one child each will have 100 per cent of the children affected when only 50 per cent were expected. In this particular example the simple expedient of omitting the fourteen probands, through whom the families were detected, corrects this bias. When this is done the ratio of affected to normal offspring becomes 9:7, which is very close to the 8:8 expected. More elaborate methods of analysis are required for pedigrees in which more than one affected offspring was independently ascertained in different families. These will be discussed in connection with autosomal recessive inheritance.

Sex-linked dominant mutations.—Sex-linked dominant mutations may be readily recognized because all the sons of affected males are normal and all the daughters are affected, while on the average half the sons and half the daughters of affected females are affected. Thus, in a pedigree of a large kindred in which some members had hypophosphatemia, Winters *et al.* (46) found that none of the ten sons and all eleven of the daughters of affected males were affected, while six of fourteen sons and seven of thirteen daughters of affected females were affected.

Sex-linked recessive mutations.—A somewhat more difficult pattern to analyze correctly, at least to judge by the confusion in the literature, concerns sex-linked recessive characters. Here again, the pattern may be readily recognized. In the case of a rare, recessive sex-linked character, affected males have normal offspring and normal parents and are related to each other via females. Fifty per cent of the sons of all women with an affected father may be expected to be affected. Normal females who have had affected sons may expect half their daughters to transmit the character. Chart 1 (from Ref. 1) presents a pedigree characteristic of a sex-linked post-natal lethal character. The pedigree concerns an as yet unnamed disease characterized by thrombocytopenia, eczematoid dermatitis, bloody diarrhea, and draining ears (1, 17, 47). The pedigree certainly appears to be due to a sex-linked recessive gene lethal early in life. A common but incorrect pro-

cedure used to quantitate this impression is to count the number of affected and normal male offspring of females related to the first known heterozygous female via females. (Other more incorrect procedures are used, but we need not concern ourselves with those.) The error in this procedure lies in the assumption that all females in the pedigree, selected as previously indicated, are equally likely to have affected sons. A correct procedure is as follows:

Starting with the proband (VI-28, Chart 1), we find that his mother (V-16), grandmother (IV-6), and great-grandmother (III-5) all must be heterozygous for the gene, for it to have reached him. IV-7 must also be heterozygous, because she is an identical twin of IV-6; half the sons of these women may be expected to be affected. The probability that a daughter of any of these women is a heterozygote is $\frac{1}{2}$ and that such a daughter, if heterozygous, will have an affected son is again $\frac{1}{2}$; hence, the probability that grandsons, of III-5, IV-6, and IV-7, descended via daughters, would be affected is $(\frac{1}{2})(\frac{1}{2}) = \frac{1}{4}$. Similar reasoning gives the probabilities for each of the females in the pedigree. The result of such calculations is that 15.25 of

CHART 1.—Pedigree showing six generations of a family in which some male infants suffer from a sex-linked recessive disease (from Aldrich, Steinberg, and Campbell [1]).

the male infants in the pedigree were expected to be affected on the basis of a sex-linked recessive gene. Fifteen were observed to be so affected.

The incorrect procedure would have shown a considerable (but in this case not significant) difference between the observed and the expected values (fifteen affected and 25 normal observed, vs. 20 of each expected).

Autosomal recessive genes.—We turn now to the most difficult of the regular patterns to analyze, namely, the autosomal recessive. Li (20) has recently reviewed the methods appropriate to the analysis of pedigrees concerned with autosomal recessive inheritance. (Unfortunately, he has followed Fisher [13] and calls what is commonly known as the *a priori* or Apert method the proband method.)

The data for studies in which recessive inheritance is suspected usually consist of a series of families composed of parents and offspring. The families may have been found in any of several different ways. The method of analysis to be used is determined by the method of ascertaining (finding) the families. In general, families involving recessive characters are ascertained via affected children. Three different patterns of ascertaining the set of families may be recognized:

a) Every individual in a community may be examined regardless of his condition or that of his relatives;

b) Some affected individuals (one or more per family) may be found by direct observation, while others may be found because they are related to affected individuals; and

c) Each family may be selected via one and only one affected child regardless of how many children in the family may be affected.

Bailey (4) has called these "complete selection of affected individuals," "multiple incomplete selection of affected individuals," and "single selection of affected individuals," respectively, or, more briefly, "complete selection," "incomplete selection," and "single selection." More recently Morton (26) has suggested the terms truncate selection, multiple selection, and single selection, respectively, for those suggested by Bailey. Morton's suggested terms will be used in what follows.

The method of analysis to be used is determined by the method of ascertaining the families. Methods appropriate for each mode of ascertainment have been developed. They are listed in Table 1.

For truncate selection the maximum likelihood method is more efficient (has a smaller variance) than the *a priori* method, but the latter is considered easier to compute because tables of

$$\frac{sp}{1-q^s} \text{ and } \frac{spq}{1-q^s} - \frac{s^2p^2q^2}{(1-q^s)^2}$$

for $p = .25$ and $.50$ are readily available (16, 30). The maximum likelihood calculation is almost as simple if Finney's tables (12) are available.

The proband method is commonly used for samples obtained by multiple selection. It is not fully efficient (13), except in the limiting case of single selection (15). Bailey (3) has developed a maximum likelihood method for analyzing data collected by multiple selection, but it is laborious to compute. Methods appropriate to samples obtained by multiple selection may be used only when the number of ascertainments is known.

When ascertainment is via single selection, the proband method becomes identical with the maximum likelihood method and is the appropriate method to use. The estimate of p and its variance are not only easy to compute, but ascertainment via single selection may yield about twice as much information per index case as complete selection (9); hence, it seems that, when feasible, this is the method of choice. *It is important that the data be analyzed by the method suitable to the mode of ascertainment. Examples will illustrate this point as well as the methods.*

Examples of the effect of ascertainment on the method of calculation.—It should be

TABLE 1

SUMMARY OF METHODS FOR ANALYZING FAMILY DATA FOR AUTOSOMAL GENES

(Based on work of Weinberg [45], Fisher [13], Haldane [15], Bailey [4], and others.)

Ascertainment	Method of analysis	Equations: Estimate	Equations: Variance
Truncate	*a priori*	$R_c = \sum_{s=2} \frac{sp}{1-q^s} n_s$	$\sum_{s=2} n_s \left[\frac{spq}{1-q^s} - \frac{s^2p^2q^2}{(1-q^s)^2} \right]$
	Maximum likelihood	$\frac{R}{p_c} = \sum_{s=2} \frac{sn_s}{1-q_c^s}$	$\frac{p_c q_c (1-q_c^s)^2}{\Sigma s (1-q_c^s - sp q_c^{s-1}) n_s}$
	Using Finney's tables	$p_c = \frac{\Sigma W Y n_s}{\Sigma W n_s}$	$\frac{1}{\Sigma W n_s}$
		Where $\Sigma W Y n_s = \frac{R}{pq} - \sum \left(\frac{s^2 p q^{s-2}}{(1-q^s)^2} n_s \right) = \frac{R}{pq} - \Sigma B n_s$	
		and $\Sigma W n_s = \frac{\Sigma s (1-q^s - sp q^{s-1})}{pq(1-q^s)^2} n_s$.	
Multiple	Proband	$p_c = \frac{\sum_{r=1} \sum_{s=2} n_{sr} a (r-1)}{\sum_{r=1} \sum_{s=2} n_{sr} a (s-1)}$	$\sum_a \frac{1}{\Sigma a} \cdot \frac{pq}{(s-1)} \times [1 + p'_a + p p'_a (s-3)]$
		where $p'_s = \frac{\sum_a \sum_r a (a-1) n_{sra}}{\sum_a \sum_r a (r-1) n_{sra}}$ = the probability of ascertainment.	
Single (minimum)	Proband (maximum likelihood)	$p_c = \frac{R-N}{T-N}$	$\frac{(T-R)(R-N)}{(T-N)^3}$ or $\frac{p_c q_c}{T-N}$

a = number independently ascertained.
s = size of family.
r = number of affected in a family.
R = total number of affected in the sample.
n_s = number of families of size s.
n_{sr} = number of families of size s with r affected.
n_{sra} = number of families of size s with r affected of which a have been independently ascertained.
N = total number of families in the sample.
T = total number of children in the sample.
p_c = computed value of p.
q = $1 - p$ = probability of not being affected.
p' = probability of being ascertained.
p = probability of being affected.

noted that in all analyses the family data must be grouped by type of mating as determined by the parents' phenotype. The matings must be grouped into those with both parents normal, those with one parent affected, and those with both parents affected. Matings of the first type are Aa × Aa (heterozygote by heterozygote) and have an expected frequency of affected offspring of .25, matings of the second type are Aa × aa with expected frequency of affected offspring equal to .50, while those of the third type are aa × aa with all offspring expected to be affected. In what follows we will consider only matings with both parents normal.

1. Truncate selection: When selection is truncate the families of different types will be represented in the sample in proportion to the frequency of its occurrence (see Li, 1954, for review and proof).

Table 2 presents a sample generated by assuming truncate ascertainment. Examples of the use of the *a priori* method are numerous and readily available (References 16, 31, 41, and others); therefore, I will not illustrate it. I will illustrate, instead, the maximum likelihood analysis using Finney's tables. This requires the selection of a preliminary value of p, the calculation of two values, and the division of one by the other. The values to be computed are shown in Tables 1 and 2. Finney (12) has published tables of $1/pq$, B, and W for a convenient series of values of p and for families of sizes two through 20. Since the data are being tested for recessive inheritance the most reasonable value of p to assume is .25. The details of the calculation are shown in Table 2.

The value for $1/pq$ with $p = .25, q = .75$, as read from Table 1 in Finney's paper (12) is 5.3333. This multiplied by 152 (the total number of affected in the sample) equals 810.662. Bn_s is obtained by multiplying the observed number of families of size s by the value of B appropriate to the value of s and the assumed value of p. The values of B are listed in Table 2 of Finney's paper. The products obtained over all values of s are summed to give ΣBn_s as shown in Table 2 of this paper.

The difference between R/pq and ΣBn_s equals ΣWYn_s. The latter divided by ΣWn_s provides the desired estimate of p. The variance of the estimate equals $1/\Sigma Wn_s$. ΣWn_s is found by reading from Finney's Table 2, values of W appropriate to s and the assumed value of p, multiplying this value of W by n_s and summing the products obtained for the different values of s. This calculation is also shown in Table 2.

The method is an iterative one, so the calculation should be repeated with the estimated value of p if it does not closely approximate the initially assumed value of p. In practice, sufficient accuracy is obtained by using a new assumed value of p which can be found in the tables. The value to be chosen is the next higher value of p to be found in the table if the originally assumed value of p is less than p_e and vice versa, the next lower value if the assumed value of p is greater than p_e. This will be illustrated in the next section.

Note that the use of a method appropriate to single selection greatly underestimates the value of p.

2. Single selection: In this case the probability of finding a family is directly proportional to the number of affected in the family. Therefore, the relative frequency with which a family of size s with r affected will be found is

$$r \frac{s!}{r!\,(s-r)!} p^r q^{s-r}.$$

This value, multiplied by the observed total number of families of size s, gives the expected number of families of size s with r affected.

The data in Table 3 were elaborated on the assumption that ascertainment was single and that all matings were Aa × Aa. The expected value of p is .25. The estimated value of p by the method appropriate to single ascertainment is of course also .25 (Table 3). The standard error is ±.04. The estimate of p by the maximum likelihood method appropriate to truncate as-

certainment with the aid of Finney's tables is also illustrated in Table 3.

The initial assumed value of p was the obvious one of .25. The estimated value of p (.401) was derived, as in the case of complete ascertainment, by Finney's tables for values of $1/pq$, B, and W, and computing the necessary products and sums. Since the estimated value differed greatly from the assumed value, the calculation was repeated using .40 for p. This gave a new estimate of p, .388. A third cycle was tried with the use of .35 for p, this being the next lower value of p in the

TABLE 2

A HYPOTHETICAL SAMPLE TO REPRESENT TRUNCATE ASCERTAINMENT OF A RECESSIVE CHARACTER

All matings were between heterozygotes

s	r	n_{sr}	n_s	i_s	R_s
2	1	42	49	98	56
2	2	7			
3	1	54	74	222	96
3	2	18			
3	3	2			
			123	320	152

Analysis: A.—Truncate ascertainment; maximum likelihood method. (All values in parentheses were read from tables in Finney, 1949.)

Assumed value of $p = .25$. $R = 152$

$$\frac{R}{pq} = 152\,(5.3333) = 810.662$$

$$-\Sigma Bn_s = -49\,(5.224) - 74\,(5.049) = -629.602$$

$$\Sigma WYn_s = \frac{R}{pq} - \Sigma Bn_s = 181.060$$

$$\Sigma Wn_s = 49\,(3.483) + 74\,(7.480) = 724.187$$

$$p_c = \frac{\Sigma WYn_s}{\Sigma Wn_s} = \frac{181.060}{724.187} = .2500$$

$$V = \frac{1}{\Sigma Wn_s} = .001381$$

$$\sigma = .037$$

B. Single ascertainment.

$$p_c = \frac{R-N}{T-N} = \frac{152-123}{320-123} = \frac{29}{197} = .147$$

$$V = \frac{(.147)\,(.853)}{197} = .000636$$

$$\sigma = .025 \qquad \frac{d}{\sigma} = \frac{.103}{.025} = 4.12$$

tables. The estimate of p was .389. Either .388 or .389 could be accepted; .388 was accepted. The value of $\Sigma W n_s$ appropriate to the estimation of the standard error of p (.388) is obtained by interpolation between the values of $\Sigma W n_s$ for $p = .35$ and $p = .40$. Note that the use of a method appropriate to complete selection has grossly overestimated p.

3. Multiple selection: Finally, for samples with known ascertainment which is neither complete nor minimum, Wienberg's proband method (13, 45) or Bailey's maximum likelihood method (3) may be used. Because such data are rarely available and the calculations to obtain a weighted mean estimate of p are extensive, I shall not detail them here. Those interested are referred to the original papers.

Haldane (15) has suggested that when ascertainment is unknown, p be estimated twice; once on the assumption of complete ascertainment (p_1) and once on the assumption of minimum ascertainment (p_0). The hypothesis of recessive inheritance is to be accepted when

$$p_1 + 2\sigma_{p_1} > .25 > p_0 - 2\sigma_{p_0} .$$

Since p_1 tends to overestimate the observed value of p if the data have not been collected by complete ascertainment, while

TABLE 3

A HYPOTHETICAL SAMPLE TO REPRESENT SINGLE ASCERTAINMENT OF FAMILIES WITH PARENTS HETEROZYGOUS FOR A RECESSIVE GENE

s	r	n_{sr}	n_s	R_s		t_s
1	1	30	30	30		30
2	1	36	48	36	60	72
2	2	12		24		24
3	1	27		27		81
3	2	18	48	36	72	54
3	3	3		9		9
	Totals:	126		162		270

Analysis: A.—Single ascertainment

$$p_c = \frac{R - N}{T - N} = \frac{162 - 126}{270 - 126} = \frac{36}{144} = .25$$

$$\sigma = \left(\frac{p_c q_c}{T - N}\right)^{1/2} = \left(\frac{(R - N)(T - R)}{(T - N)^3}\right)^{1/2} = \left(\frac{(.25)(.75)}{144}\right)^{1/2} = .0361 .$$

B.—Truncate ascertainment

1st cycle: $p = .25$ $R = 132$
$\Sigma W Y n_s = 210.892$
$\Sigma W n_s = 526.224$
$p_c = .401$

2nd cycle: $p = .40$ $R = 132$
$\Sigma W Y n_s = 193.844$
$\Sigma W n_s = 499.824$
$p_c = .388$

3rd cycle: $p = .35$ $R = 132$
$\Sigma W Y n_s = 191.947$
$\Sigma W n_s = 493.920$
$p_c = .389$
accept = .388
$\Sigma W n_s$ for .388 by interpolation = 498.407

$$\sigma_{p_c} = \left(\frac{1}{498.407}\right)^{1/2} = .045$$

p_0 tends to underestimate the observed value of p if the data have not been collected by minimum ascertainment, I have often wondered if Haldane's criterion is not too broad.

It seems to me to be highly unlikely that data collected by truncate ascertainment will not be known to have been so collected; consequently, p_1 will always overestimate p. Similarly, it is unlikely, but perhaps not as unlikely as in the case of truncate ascertainment, that data collected by single ascertainment will not be recognized to have been collected in this way; consequently p_0 will always underestimate p. For these reasons a more appropriate criterion may be

$$p_1 + \sigma_{p_1} > .25 > p_0 + \sigma_{p_0} .$$

This problem is being investigated further (Littell and Steinberg, unpublished).

Among the major limitations of the methods reviewed in the foregoing are that, in general, they are suitable only for alleles with essentially complete penetrance, which have a frequency considerably greater than the mutation rate and which determine a character for which there are relatively few phenocopies. Most characters of interest in human biology are not determined by genes which satisfy these criteria. Morton (23) has pointed out that the traditional statistical methods, "... fail to provide tests of internal consistency or to distinguish among alternative hypotheses which in principle are quite distinct, such as partial sex linkage and sex-biased manifestation, or mutations, phenocopies, and incomplete penetrance. Further progress in many aspects of human genetics must await the application, not only of better laboratory techniques, but also of a more refined methodology, made possible by modern developments in mathematical statistics."

Morton (24) is actively engaged in providing methods which promise to overcome some of the deficiencies of the older methods. These methods provide a test of the internal consistency of the data and an estimate of the frequency of phenocopies. For sex-linked genes they provide in addition a method for comparing the mutation rate in males with that in females.

In essence his procedure involves (*a*) the computing and comparison of three independent estimates of the probability of an affected individual being ascertained; this tests the independence of the ascertainments—the derived probability of ascertainment may then used in maximum likelihood methods to test the mode of inheritance; and (*b*) an estimate, based on the distribution of isolated and familial cases, of the proportion of the isolated cases which may be phenocopies. The method is designed to be applied to samples which have been obtained by multiple incomplete selection and *for which the enumeration of the individuals ascertained and the number of times each has been ascertained, are accurately known and reported.*

The development and extension of these methods offer an opportunity to study entirely new types of characters in man and to study former problems with much greater precision than was previously possible.

GENE FREQUENCY

Cotterman (7), in his fine summary of methods of estimating allele frequencies at a single locus, summarizes the uses of such estimates as follows:

1. comparing two or more populations, differing in geographical location, age composition, breed, sex, etc.;

2. testing homogeneity of a single sample, i.e., testing consistency of the genetic hypothesis and sampling theory in relation to the particular sample used in estimating the gene frequencies; and

3. analyzing other bodies of data, especially collections of family records, where the expectations in various offspring classes are functions of the gene frequencies.

To these we may add:

4. as a basic datum in the estimation of mutation rates and

5. in epidemiology, to estimate the po-

tential frequency of a disease with variable age at onset or incomplete penetrance.

The methodology of gene frequency estimates is enormous and cannot possibly be adequately discussed in a single session. My discussion will be more limited than that of Cotterman cited above. Those who wish further information are referred to his paper and to the excellent book by C. C. Li (21).

In what follows we will assume that the sample consists of randomly selected, unrelated (at least not closely related) individuals, all showing the same phenotype, which in turn is assumed to be due to an allele at one locus. We shall assume further that all estimates of gene frequency, with the exception of two allele systems without dominance such as the MN system, are based on the Hardy-Weinberg equilibrium (40) or some modification of it.

The Hardy-Weinberg equilibrium occurs in a randomly mating population (i.e., a population in which mate selection is uninfluenced by the character in question), in which all pertinent genotypes are on the average equally viable and equally fertile. In such a population if p equals the frequency of the allele A and q equals $(1 - p)$ equals the frequency of the allele a, the frequencies of the genotypes are the terms of the expansion of $(p + q)^2$, namely, p^2 (AA), $2pq$ (Aa), and q^2 (aa). In general, if there are n alleles with frequencies p_1, $p_2, \ldots p_1 \ldots p_n$, the frequencies of the several phenotypes are in accord with the expansion of $(p_1 + p_2 + \ldots p_n)$.[2] As a specific example consider the ABO alleles. The frequency of A is usually represented by p, of B by q, and O by r. The genotypes are distributed as shown in Table 4. Methods for estimating gene frequencies for some specific patterns of single gene inheritance will be demonstrated in the following paragraphs.

Two alleles with no dominance or incomplete dominance.—In this type of inheritance the heterozygote may be distinguished from either hemozygote. Some familiar examples are the MN blood type system, sickle-cell trait and disease, and Cooley's trait and disease. The three genotypes may be represented by AA, Aa and aa. Table 5A summarizes the method of estimating the gene frequencies. These estimates are maximum likelihood estimates and therefore have minimum errors of estimate in large samples. The use of these estimates to test whether the population is in a Hardy-Weinberg equilibrium is shown in Table 5B. Table 6 presents a numerical illustration of the foregoing based on unpublished data for the MN blood types of 343 unrelated individuals. The χ^2 test is used to test for goodness of fit with the expected distribution based on the Hardy-Weinberg equilibrium. (Those unfamiliar with this test will find it described in any elementary text-book on statistics.) There is only one degree of freedom, despite the three comparisons, because two restrictions have been placed on the data, namely, the fixed total and the estimate of p. Since $p + q = 1$, when p has been estimated q has also been estimated. Note that in this type of inheritance the estimate of the gene frequency is accurate regardless of the distribution of the blood types. The procedure is essentially the same in situations with three or more alleles, none of which show dominance.

Dominant alleles.—Here the three genotypes lead to only two phenotypes, the heterozygotes being indistinguishable from one of the homozygous classes—i.e., AA and Aa are phenotypically indistinguishable. The two phenotypic classes in a population in equilibrium occur with frequencies $q^2(aa)$ and $(1 - q^2)(Aa + AA)$. The frequency of allele $a = q = (q^2)^{1/2}$ and the frequency of allele $A = p = (1 - q)$. The standard error of the estimate $= [(1 - q^2)/4T]^{1/2}$ where $T =$ the number of individuals in the sample. As an example we may take the data of Ikin *et al.* (quoted by Mourant [26], p. 366) on the frequency of the blood type P-positive in a sample of English people. P-positive individuals are believed to be PP or Pp, while P-negative individuals are assumed to be pp, hence P-negative may be considered recessive. Ikin *et al.* found

23.41 per cent of 1166 tested individuals to be *P*-negative (i.e., recessive homozygotes). The frequency of p is therefore $(.2341)^{1/2}$ or .4839. The frequency of $P = 1 - q = .5161$. The standard error of

$$\sigma_p = \left(\frac{1 - q^2}{4T}\right)^{1/2} = \left(\frac{1 - 0.2341}{4\,(1166)}\right)^{1/2} = \pm .0128\,.$$

Note, that in this example we cannot test the population against the Hardy-Weinberg distribution, because we have placed two restrictions on the sample (fixed total and fixed p [p^2]), and there are only two comparisons. The estimate of the frequency of p may, however, be used for comparison with estimates derived from other populations. Brendemoen (6) found the frequency of the allele p to be .5013 ± .0129 among 1162 Norwegians. We may ask, is the frequency of p among Norwegians and English statistically the same? The difference and its standard error are .0174 ± .0180; hence, the difference is not significant.

Sex-linked genes.—The estimate of gene

TABLE 4

EQUILIBRIUM (HARDY-WEINBERG) DISTRIBUTION OF THE *A*, *B*, *O*, GENOTYPES WITH FREQUENCIES OF ALLELES A, B, AND O EQUAL TO p, q, AND r, RESPECTIVELY $(p + q + r)^2$

Blood group	A	B	AB	O
Genotype	*AA* and *AO*	*BB* and *BO*	*AB*	*OO*
Frequency	p^2+2pr	q^2+2qr	$2pq$	r^2

TABLE 5

ESTIMATE OF GENE AND GENOTYPE FREQUENCIES, NO DOMINANCE

A. Estimate of gene frequencies

Genotype	*AA*	*Aa*	*aa*	Total
Observed number	G	H	I	T
Frequency = (no./total)	g	h	i	1

$$\text{Frequency of } A = \mathrm{p} = \frac{2\mathrm{g}+\mathrm{h}}{2} = \mathrm{g}+\tfrac{1}{2}\mathrm{h}$$

$$a = \mathrm{q} = \frac{\mathrm{h}+2\mathrm{i}}{2} = \tfrac{1}{2}\mathrm{h}+\mathrm{i}$$

$$\sigma = (\text{Standard error}) = \left(\frac{pq}{2\mathrm{T}}\right)^{1/2}$$

B. Estimate of genotype frequencies

Genotype	*AA*	*Aa*	*aa*	Total
Frequency	p^2	2pq	q^2	1
Number	$\mathrm{T}\mathrm{p}^2$	T(2pq)	$\mathrm{T}\mathrm{q}^2$	T

TABLE 6

EXAMPLE OF THE ESTIMATION OF THE FREQUENCY OF ALLELES WITH NO DOMINANCE

(Unpublished data for the MN groups of 343 unrelated individuals.)

GENOTYPE	*MM*		*MN*		*NN*		TOTAL	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Observed	119	34.7	155	45.2	69	20.1	343	100
Expected	112.6	32.8	167.9	48.9	62.5	18.3	343	100

$\lambda^2_1 = 2.031$ $\quad P > .10\,.$

frequency is simplified for sex-linked recessive genes, because males have but one allele; and, hence, if penetrance is complete, the phenotype corresponds to the genotype. The frequency of a sex-linked allele may be estimated, therefore, by simply determining the proportion of males in the population who show the phenotype associated with that allele. The only two sex-linked genes that I know of for which this has been done are color-blindness and hemophilia. Unfortunately, neither of these fulfills the requirement that the phenotype be determined by a single genotype. Sex-linked color-blindness is due to at least two different genes, and so is sex-linked hemophilia. The latter is divided into classic hemophilia (AHF deficiency) and Christmas disease (or PTC deficiency). We may use the hemophilia data as an example, however, and following Graham (14) correct for the relative frequency of the two types of sex-linked hemophilia as determined by studies on relatively unselected groups of hemophiliacs.

TABLE 7

FREQUENCY OF HEMOPHILIA AND CHRISTMAS DISEASE

After Graham, 1956

	No.	Per cent
Total cases surveyed and reclassified:	247	100
Hemophilia	207	83.8
Christmas disease	40	16.2
Frequency of hemophilia in Danish males (Andereasson's survey)		4.45×10^{-4}
Adjusted frequencies for Danish males:		
Hemophilia		3.73×10^{-4}
Christmas disease		0.72×10^{-4}

Table 7 taken from Graham (14) illustrates the procedure. Other examples and applications will be found in Cotterman's review (7).

Use of consanguinity among parents to estimate gene frequency.—Various authors have derived expressions for estimating the frequency of a rare recessive gene by using the frequency of consanguineous marriages among the parents of affected individuals and among the general population. The only formulae likely to be of practical use are those for first-cousin marriages. The derivation of these and others is reviewed by Dahlberg (8, pp. 60–63).

If c_1 = the frequency with which first-cousin marriages occur,
c_2 = the frequency expected in panmixia,
q = the frequency of the recessive allele,
and k = the frequency of first-cousin marriages among the parents of affected individuals, then

$$k = \frac{c_1(1+15q)}{(c_1-c_2)(1+15q)+16q\,[1-(c_1-c_2)]}. \qquad (1)$$

If first-cousin matings occur with the expected frequency ($c_1 = c_2$),

$$k = \frac{c_1(1+15q)}{16q}. \qquad (2)$$

If the population is very large so that $c_2 \to 0$,

$$k = \frac{c_1(1+15q)}{c_1(1+15q)+16q(1-c_1)} = \frac{c_1(1+15q)}{c_1+16q-c_1q}, \qquad (3a)$$

and

$$q = \frac{c_1(1-k)}{16k-15c_1-c_1k}. \qquad (3b)$$

Note the conditions on which equations (3a) and (3b) are based, namely:

a) All occurrences of the character are genetically determined and due to the same allele;

b) the gene frequency is uniform throughout the population being sampled;

c) matings occur essentially at random;

d) all genotypes, with respect to the locus, are equally fertile on the average;

e) the expected frequency of first-cousin

marriages is essentially zero.

Assumption (*a*) is inherent in all gene frequency estimates, assumptions (*b*), (*c*), and (*d*) are assumed in all estimates of recessive genes, while (*e*) is pertinent only to the present method. This procedure requires an estimate of the frequency of first-cousin marriages in the general population and among the parents of a properly selected sample of affected individuals. It does not, however, require an estimate of the frequency of affected individuals in the population.

Although there are many sources of error in gene frequency estimates based on this equation, such estimates can provide orders of magnitude of the frequency of various genes when other preferred methods are not available. An example of the use of this method based on equation (3*b*) may be found in the paper by Neel *et al.* (30).

Average frequency of a class of mutations.—A major source of error in estimates of gene frequencies is that two or more different genes may lead to the same phenotype or to phenotypes that are difficult to distinguish. A second difficulty associated with the procedures outlined above is that only genes leading to clearly distinguished phenotypes due to simple genetic patterns may be studied. These genes compose but a very small minority of the total. Yet fundamental problems such as those having to do with genetic damage in the sense of wasted pregnancies, early post-natal deaths and generally reduced efficiency require estimates of the frequencies of large numbers of genes which, individually, do not lead to easily distinguished phenotypes. A method for estimating the average frequency of a group of such genes would indeed be highly desirable.

Muller (27), by an ingenious indirect method, estimated the average mutation rate per human gamete, the average detrimental effects of these mutations, and their average dominance. Using these estimates which were 0.1 new mutations per gamete, with an average detrimental effect of 2.5 per cent and an average dominance of 5 per cent, Muller estimated that each individual, on the average, carries eight slightly dominant, detrimental mutant genes in heterozygous condition. Detrimental effect here means the average reduction in fertility (the number of offspring surviving to reproduce) caused by the gene in the homozygous condition; average dominance refers to the average reduction in fertility caused by the gene in the heterozygous condition divided by that caused by the gene in the homozygous condition (i.e., detrimental effect when heterozygous/detrimental effect when homozygous.)

As Muller pointed out, more direct methods of determining the average frequency of detrimental genes in the population are desirable.

S. C. Reed (35) indicated that the offspring of father-daughter or brother-sister matings could be used to estimate the frequency in the population of heterozygosis for a group of known recessive genes. The procedure rests on the genetic fact that the probability that a daughter will carry a recessive gene for which her father or brother is heterozygous is one-half. Hence, the probability that a father-daughter mating would yield a child homozygous for a recessive gene present in the father is one in eight (i.e., the probability that she is heterozygous [$\frac{1}{2}$] times the probability of a homozygous recessive offspring from the mating of two heterozygotes). It is possible with this procedure to compute the average number of genes for which the father is heterozygous as a function of the frequency with which such offspring are homozygous for recessive genes.

Slatis (38) extended the method to first-cousin marriages which are probably more frequent than incestuous matings. He provided a method for taking family size into account and for combining data from families of different sizes. His method is as follows:

Let s = size of family;

$z_s = 1 - (\frac{3}{4})^s$ = the probability of at least one affected child in a

family of size s with heterozygous parents,

r_s = the number of recessive genes in families of size s,

n_s = the number of families size s.

and c = the average number of recessive genes for which the parents of these families are heterozygous.

Then $z_s c$ mutants will be seen per family of size s, and $n_s z_s c = r_s$, mutants will be seen in n_s families of size s, hence

$$c = \frac{r_s}{n_s z_s}.$$

In the total sample, the families of various sizes will have an average of

$$\bar{c} = \frac{\Sigma_s r_s}{\Sigma_s n_s z_s} \text{ mutants per family .}$$

The average number of genes for which *each pair* of common grandparents is heterozygous equals $16(c)$, because the probability that two first cousins will each inherit the same gene from among the four alleles in the common grandparents is 1 in 16. Hence, *each* of the common grandparents is heterozygous for $8(c)$ genes. Penrose (33) applied a similar method of analysis to data collected by Böök (5) in a North Swedish population. The correct estimate from these data is that each individual is heterozygous for about six mutants. This estimate is lower than Muller's, but it considers only major deviations known or suspected to be due to recessive genes.

Morton, Crow, and Muller (25), have extended the method to provide an estimate of the number of "lethal equivalents" present in the heterozygous condition in the average person. The lethal equivalent value of an allele equals the value of the selection coefficient (s) against the homozygote for that allele. Thus, an allele which causes death in all homozygotes (s equals one) is said to equal one lethal equivalent. An allele which causes death in half the homozygotes ($s = .5$) is said to equal .5 of a lethal equivalent. The actions of the several genes are assumed to be independent (non-synergistic [27]), but their effects are assumed to be additive. Hence, a gamete with two mutants, each of which would cause death in 50 per cent of those zygotes homozygous for it, may be said to contain one lethal equivalent.

In essence, the method developed by Morton *et al.* (25) consists of dividing the causes of death into two portions: One, called '*A*', represents deaths due to environmental conditions plus those due to homozygosity and heterozygosity resulting from non-consanguineous matings; while the other, called '*B*', equals deaths due to homozygosity resulting from inbreeding minus the genetic deaths represented by '*A*.' '*A*' and '*B*' are evaluated as follows:

Let

F = the coefficient of inbreeding (48). It is the probability that both alleles at a locus have been derived from one present in a common ancestor; that is, they are identical by descent (Li and Sacks, 1954);

s = the selection coefficient against the homozygote; that is, the proportionate reduction in the relative fertility of homozygotes;

h = a measure of dominance; it is the fraction of the selection coefficient(s) expressed against heterozygotes;

S = the probability of surviving.

Sewall Wright (48) has shown that, in a population with an inbreeding coefficient F, the three genotypes for a given locus occur with the following frequencies (see Li [21], chapter 11 for review):

Genotype	$A\,A$	$A\,a$	$a\,a$
Frequency	$F(1-q) + (1-F)(1-q)^2$	$2(1-F)\,q\,(1-q)$	$Fq + (1-F)\,q^2$

Where $F(1-q)$ and Fq equal the frequencies of homozygosis because of inbreeding, for the dominant and recessive alleles, respectively, and the remaining terms represent the frequencies of the indicated genotypes which occur in the absence of inbreeding.

Therefore, the probability that an allele with frequency q will cause death in a population with a coefficient of inbreeding equal to F, selection pressure against homozygotes for the allele equal to s, and against heterozygotes for this allele equal to hs is

$$\underset{\text{homozygotes}}{qFs+q^2(1-F)\,s}+\underset{\text{heterozygotes}}{2q(1-q)(1-F)\,sh}\ ,$$

where the relative survival value of the homozygous dominant is one.

The probability of surviving genetic causes of death is one minus the above quantity, or

$$1-qFs-q^2s+q^2Fs-2q(1-q)\,sh + 2q(1-q)Fsh\ .$$

Death may occur for non-genetic (environmental) reasons. Let the probability of death due to a particular environmental cause be x, and be independent of the genotype. The probability of not succumbing to environmental causes (surviving) is therefore $(1-x)$. The probability of surviving despite a given environmental risk and the genetic risk due to a specific allele is therefore:

$$(1-x)\,[1-qFs-q^2s+q^2Fs - 2q(1-q)\,sh+2q(1-q)Fsh]\ ,$$

which on expansion equals

$$1-qFs-q^2s+q^2Fs-2q(1-q)\,sh + 2q(1-q)Fsh-x\ ,$$

plus a series of terms involving the product of x and the terms other than one in the bracket. Since x and each of these terms are small, their products may be ignored without introducing great error into what is at best an approximate calculation.

The probability of dying is one minus the above quantity or

$$x+q^2s+2q(1-q)\,sh+qFs-q^2Fs - 2q(1-q)Fsh\ .$$

The over-all probability of death from any environmental cause or any locus is the sum of these terms and

$$=\Sigma x+\Sigma q^2s+2\Sigma q(1-q)\,sh + F[\Sigma qs-\Sigma q^2s-2\Sigma q(1-q)\,sh]\ .$$

Morton *et al.* (23), let:

$$A=\Sigma x+\Sigma q^2s+2\Sigma q(1-q)\,sh\ , \quad \text{and}$$

$$B=\Sigma qs-\Sigma q^2s-2\Sigma q(1-q)\,sh\ .$$

Hence, the probability of death from some cause may be written as

$$A+BF$$

The first term of the Poisson distribution expresses the probability that an event with a given mean expectancy (in this case $A+BF$) will not occur. The event we are discussing is death. If death does not occur, survival does, hence the probability of survival (S) may be stated as the first term of a Poisson distribution and

$$S=e^{-(A+BF)} \quad \text{or} \quad -\log_e S=A+BF$$

The value of F is computed for each degree of consanguinity observed in the sample. The survival rate is observed for the offspring resulting from marriages of each degree of consanguinity. These values are used to estimate the values of A and B. Note that B is less than Σqs, the mutational damage per gamete, and that $A+B=\Sigma qs+\Sigma x$ is greater than the Σqs. Hence, the number of lethal equivalents per gamete is somewhat less than $A+B$ and more than B.

Using data from three different sources, which among them included data on stillbirths and post-natal deaths up to age 20,

Morton, Crow, and Muller estimated that the average individual is heterozygous for three to five lethal equivalents. The authors believe their estimate is probably too low, because the data omit abortions, early adult deaths, and cases of infecundity. Since a lethal equivalent may comprise two or more detrimental mutants, each individual is, on the average, probably heterozygous for more than five genes. Morton *et al.* (23) computed also that the average person carries an additional four to five genes which, if homozygous, could cause conspicuous abnormality and probably reduced reproductive potential.

MUTATION RATES

The mutation rate of a specific dominant gene may be estimated by determining the incidence of affected individuals whose parents were not affected. This method, which is known as the direct method, assumes complete penetrance of the dominant gene. If penetrance is incomplete, a correction based on an estimate of the probability that a phenotypically normal individual heterozygous for the gene will have had only one affected child among '*s*' offspring must be introduced. In either situation it is assumed that all cases are due to the same locus and, incidentally, that all are offspring of their legal parents.

Alternately, the mutation rate may be estimated by the indirect method which may also be used to estimate the mutation rate of recessive alleles. All indirect methods of estimating mutation rates depend upon the assumption that the population is in equilibrium, i.e., that the frequency of the allele in question remains constant from generation to generation. If this assumption is correct, the frequency of origin of the allele by mutation must equal the frequency of loss of the allele by reduced fertility of a portion of those carrying it (see Muller [27] for a detailed discussion). Additional assumptions are: (*a*) all instances of the character in question are genetically determined and due to the same locus; and (*b*) for recessive alleles, mating is at random with respect to the locus in question.

Table 8, modeled after the one published by Penrose (32), presents a summary of the indirect methods for estimating the mutation rates of autosomal dominant and recessive genes and sex-linked recessive genes (see Stern [41], pp. 408–12, for lucid and simple derivations of these methods). It should be noted that all methods ignore reverse mutations, i.e., if the allele under consideration is dominant (*A*), mutation from '*a*' to '*A*' is examined, but mutation from '*A*' to '*a*' is not.

TABLE 8

SUMMARY OF INDIRECT METHODS FOR ESTIMATING THE MUTATION RATES OF INDIVIDUAL GENES

Steps in calculation	Rare dominant trait	Sex-linked trait	Recessive trait
1. Sex affected	M or F	M	M or F
2. Genotype of affected	Aa	a	aa
3. Frequency of genotype in population	$2p$	q	q^2
4. Relative loss of fitness	$(1-f)$	$(1-f)$	$(1-f)$
5. Mutation rate/gamete to allele causing genotype	$(1-f)p$	$\frac{1}{3}(1-f)q$	$(1-f)q^2$

Step three (Table 8) indicates that it is necessary to determine the frequency of affected individuals in the general population. This involves an enumeration of all affected individuals in a given region and an enumeration of the total population of which they form a part. There is room for error in both these enumerations. The numerator (the number of affected) may be underestimated because some diagnosed cases were missed, or because some cases have not been diagnosed, or have been misdiagnosed, and so on. It may be overestimated if some cases are not genetically determined or are due to different genes. Errors in

the denominator (size of the total population) may result from any one of several reasons, among the most important are the use of an incorrect base population (in time or location, or both), and faulty census data. Neel (29) has reviewed and illustrated these difficulties with respect to three dominantly inherited, rare diseases.

The frequency of affected individuals is used to estimate the frequency of the allele in the population. In a population mating at random, the frequency of the heterozygote is $2pq$. Since $q = (1 - p)$ we may write this as $2p(1 - p)$ or $2(p - p^2)$. In the case of a rare dominant p is small (less than .001), hence p^2 is very small. Therefore $2p(1 - p)$ is very nearly equal to $2p$. Step 4 indicates that the relative fertility (f) of affected individuals must be determined. In practice this is based on the average number of surviving offspring per affected individual divided by the average number of offspring per unaffected individual of a comparable socio-economic, ethnologic, etc., group. Some aspects of the problems of estimation involved here have been reviewed by Krooth (19), and Reed and Neel (36).

If f is the relative reproductive fitness of affected individuals, $(1 - f)$ is the relative loss in reproductive fitness of such individuals. For every individual lost one dominant gene is lost. This lost gene must be replaced by one of the two genes which give rise to a new individual; hence, the rate of replacement of genes required for equilibrium equals $(1 - f)p$ (recall that $2p$ equals the frequency of dominant individuals).

We shall consider the estimate of the mutation rate of the gene causing retinoblastoma as an example of the method of estimating the mutation rate of a dominant gene and of the pitfalls awaiting even highly skilled workers well aware of the sources of error. In a very careful study of retinoblastoma in Michigan, Falls and Neel (11), using the direct method, estimated the mutation rate of the normal allele causing retinoblastoma as 2.3×10^{-5}. This estimate, as the authors pointed out, was based on the assumption that all retinoblastoma is genetically determined and due to the same gene with essentially complete penetrance. A mutation rate of 1.6×10^{-5} may be derived by using the authors' data for the incidence of the disease and their estimate of the relative reproductive fertility of retinoblastoma parents. This is not very different from the rate derived by Philip and Sorsby (34) from data collected in London, namely, 1.4×10^{-5}. As we shall see, all these estimates are probably too high. Vogel (43), from a consideration of the proportion of cases which are hereditary (i.e., have affected ancestors) and the relative fertility of retinoblastoma patients, concludes that only 25 per cent of all sporadic cases represent mutations, the remaining 75 per cent being phenocopies (environmentally induced non-hereditary copies of phenotypes which are known to be genetically determined) or somatic mutations.

Tucker, Steinberg, and Cogan (42) and Vogel (44) independently checked this point by determining the frequency of retinoblastoma among the offspring of individuals who had survived the disease and who had no known collateral or ancestral relatives with retinoblastoma. These direct studies indicate that about 20 per cent of the sporadic cases are due to new mutations; the remainder appear to be non-genetic. Vogel (44) estimates the mutation rate to be 6 to 7×10^{-6}, i.e., about one quarter the original estimate of Falls and Neel (11) and about half the rate estimated by Philip and Sorsby (32).

These several papers serve to illustrate, forcibly, the difficulties confronting those who would study mutation rates and the necessity for caution in comparing estimates of the mutation rates of specific genes computed for different populations.

We have already discussed the difficulty encountered in studying the frequency of the gene causing hemophilia, namely, that clinical hemophilia has been shown in the laboratory to be a group of diseases. Genetic evidence shows that two of these, classical hemophilia (AHF deficiency) and Christmas disease (plasma thromboplastin component, PTC, deficiency), are due to sex-

linked genes (review in Graham [14]). Clearly, the earlier calculations of the mutation rate for hemophilia will have to be reevaluated. It is not all certain, however, that all cases of AHF deficiency are due to the same allele; hence, even the separation of sex-linked hemophilia, into the two categories of AHF deficiency and Christmas disease, will not insure an accurate estimate of the mutation rate.

The estimate of the mutation rate for an autosomal recessive mutant has, in addition to the complications already enumerated and illustrated, the difficulty that the gene may not be completely recessive and that the survival value of the heterozygote may not be identical with that of the homozygous normal. This can introduce considerable error into the estimate, because most of the recessive alleles in the population will be carried by heterozygotes, when the recessive allele is less frequent than the dominant allele. For practical purposes this is true of all diseases for which mutation rates would be calculated. As an illustration, consider a relatively frequent recessive disease, one with a frequency of .01. About nine times as many recessive alleles would be carried by heterozygotes as by homozygotes. Hence, any deviation from complete recessivity may be expected to have a marked influence on the mutation rate required to maintain the population at equilibrium for such an allele. If the heterozygote is more viable than either homozygote, a situation postulated for the sickle-cell trait (see Allison [2] for review), a lower rate of mutation is required to maintain equilibrium; if the heterozygote is less fit than the homozygous dominant, a higher mutation rate is required to maintain equilibrium. These problems have been recognized and discussed by many authors. A relatively recent discussion of them may be found in a paper by Neel (28).

An alternative to estimating the mutation rate of specific genes is to estimate the average mutation rate of a group of genes causing a reduction of fitness of those carrying them. Morton, Crow, and Muller (25) showed that the method they developed to estimate the average number of such genes carried by an individual offers a means of doing this. It is assumed, as in all indirect estimates of mutation rates, that the population is at equilibrium with respect to the frequency of these alleles. Morton *et al.* proceeded as follows:

Let Fs = the probability of a given mutant's being eliminated through homozygosity resulting from inbreeding;

$(1 - F)qs$ = the probability of a given mutant's being eliminated through homozygosity resulting from its meeting a previously existing allele;

$(1 - F)(1 - q)sh$ = the probability of a given mutant's being eliminated in the heterozygous condition.

The total probability of elimination is the sum of these probabilities or approximately $Fs + qs + sh$ (terms such as Fqs, Fsh, and $Fqsh$ being omitted, because they are small compared with those retained). The above expression may be written as $(F + q + h)s$, and by designating $F + q + h$ as z; as zs. If zs is the probability that a mutant allele will be eliminated in any given generation, the average number of generations it will exist in the population before it is eliminated (the mean persistence) equals $1/zs$. The frequency of the allele in the population (i.e., the probability of its occurring in a gamete) will equal μ/zs, where μ is the mutation rate. Recall that s equals the fraction of a lethal equivalent represented by a given allele. Then an allele with frequency μ/zs and a lethal equivalent value of s will contribute $(\mu/zs)s$, or μ/z lethal equivalents per gamete. The number of lethal equivalents contributed by all alleles may be found by summing over all loci; i.e., the total number of lethal equivalents per gamete equals $\Sigma(\mu/z)$. Under certain conditions $\Sigma(\mu/z)$ may be written as $(\Sigma\mu)(\overline{1/z})$ (23). Hence, the total mutation rate per gamete $(\Sigma\mu)$ may be estimated from the estimate of the number of lethal equiv-

alents per gamete if a value for $\overline{1/s}$ (the harmonic mean of s) can be obtained. Using data from Drosophila, Morton *et al.* (25) consider .02 to be a reasonable estimate of the harmonic mean of s. Using this value and the previously derived value of 1.5 to 2.5 lethal equivalents per gamete, they arrived at total mutation rate of alleles causing death between late fetal and early adult life of .03 to .05 per gamete per generation. The total detrimental mutation rate, including that of genes causing early embryonic death, is estimated to be 2–3 times as great, i.e., from .06 to .15 per gamete.

This method promises to be most useful in determining gene frequencies and mutation rates. It seems to be the best method for obtaining the data required to evaluate the effect of mutagens on man. Better and more extensive data based on human material are required for each of the applications. We are all obligated to help supply such data whenever we can.

Only some of the more important or more commonly used methods for the analysis of hereditary patterns, the determination of gene frequencies and of mutation rates have been discussed. There has not been time to discuss such important problems as the measurement of linkage, the determination and measurement of factors influencing the frequencies of genes in populations, and so on. The interested reader will find references to literature on these topics in *The American Journal of Human Genetics*.

It has been my purpose to expose you to some of the patterns of thought and methods of approach used by geneticists in solving problems of heredity in man. If this paper has opened the door to these problems for you, I am pleased; if in addition it has given you some insight into how these problems are handled, I have accomplished my purpose, and I am delighted.

REFERENCES

1. ALDRICH, R. A.; STEINBERG, A. G.; and CAMPBELL, D. C. Pedigree Demonstrating a Sex-linked Recessive Condition Characterized by Draining Ears, Eczematoid Dermatitis and Bloody Diarrhea. Pediatrics, **13**:133–39, 1954.
2. ALLISON, A. C. Aspects of Polymorphism in Man. Cold Spring Harbor Symp. Quant. Biol., **20**:239–55, 1955.
3. BAILEY, N. T. J. The Estimation of the Frequencies of Recessives with Incomplete Multiple Selection. Ann. Eugen., **16**:215–22, 1951.
4. ———. Classification of Methods of Ascertainment and Analysis in Estimating the Frequencies of Recessives in Man. *Ibid.*, pp. 223–25.
5. BÖÖK, J. A. Genetical Investigations in a North Swedish Population. The Offspring of First-Cousin Marriages. Ann. Human Genet., **21**:191–221, 1957.
6. BRENDEMOEN, O. J. Blood Factor P in Eastern Norway. Acta path. microbiol. Scandinav., **27**:338–39, 1950.
7. COTTERMAN, C. W. Estimation of Gene Frequencies in Nonexperimental Populations. *In:* KEMPTHORNE, BANCROFT, GOWEN, and LUSH (eds.), Statistics and Mathematics in Biology. Ames, Iowa: The Iowa State College Press, 1954.
8. DAHLBERG, G. Mathematical Methods for Population Genetics. New York: Interscience Publishers, 1948.
9. DAVID, P. R. *In:* Conference on Problems and Methods in Human Genetics. Am. J. Human Genet., **6**:51–194, 1954.
10. DAVID, P. R., and SNYDER, L. S. Genetics and Disease. *In:* Proc. Second Nat. Cancer Conf., pp. 1128–38. New York, Am. Cancer Soc., 1954.
11. FALLS, H. F., and NEEL, J. V. Genetics of Retinoblastoma. Arch. Ophthalmol., **46**: 367–89, 1951.
12. FINNEY, D. J. The Truncated Binomial Distribution. Ann. Eugen., **14**:319–28, 1949.
13. FISHER, R. A. The Effect of Methods of Ascertainment upon the Estimation of Frequencies. Ann. Eugen., **6**:13–25, 1934.
14. GRAHAM, J. B. Biochemical Genetics of Blood Coagulation. Am. J. Human Genet., **8**:63–79, 1956.
15. HALDANE, J. B. S. The Estimation of the Frequencies of Recessive Conditions in Man. Ann. Eugen., **8**:255–62, 1938.
16. HOGBEN, L. An Introduction to Mathematical Genetics. New York: W. W. Norton & Co., Inc., 1946.

17. HUNTLEY, C. C. and DEES, S. C. Eczema Associated with Thrombocytopenic Purpura and Purulent Otitis Media. Pediatrics, **19**:351–61, 1957.
18. IKIN, E. W.; KOPEC, A. C.; MOURANT, A. E.; PARKIN, D. M.; and WALBY, J. A. B., 1952. Unpublished data quoted in Mourant, 1954.
19. KROOTH, R. The Use of the Fertilities of Affected Individuals and Their Unaffected Sibs in the Estimation of Fitness. Am. J. Human Genet., **7**:325–60, 1955.
20. LI, C. C. Segregation of Recessive Offspring. Methods Med. Research, **6**:3–16, 1954.
21. ———. Population Genetics. Chicago, Ill.: University of Chicago Press, 1955.
22. LI, C. C., and SACKS, L. The Derivation of Joint Distribution and Correlation between Relatives by the Use of Stochastic Matrices. Biometrics, **10**:347–60, 1954.
23. MORTON, N. E. Segregation Analysis in Human Genetics. Science, **127**:79–80, 1957.
24. ———. Genetic Tests under Incomplete Ascertainment. Am. J. Human Genet., **11**: 1–16, 1959.
25. MORTON, N. E.; CROW, J. F.; and MULLER, H. J. An Estimate of the Mutational Damage in Man from Data on Consanguineous Marriages. Proc. Nat. Acad. Sc., **42**:855–63, 1956.
26. MOURANT, A. E. The Distribution of the Human Blood Groups. Springfield, Ill.: Charles C Thomas, 1954.
27. MULLER, H. J. Our Load of Mutations. Am. J. Human Genet., **2**:111–76, 1950.
28. NEEL, J. V. The Study of Human Mutation Rates. Am. Nat., **86**:129–44, 1952.
29. ———. Problems in the Estimation of the Frequency of Uncommon Inherited Traits. Am. J. Human Genet., **6**:51–60, 1954.
30. NEEL, J. V.; KODANI, M.; BREWER, R.; and ANDERSON, R. C. The Incidence of Consanguineous Matings in Japan. With Remarks on the Estimation of Comparative Gene Frequencies and the Expected Rate of Appearance of Induced Recessive Mutations. Am. J. Human Genet., **1**:156–78, 1949.
31. NEEL, J. V., and SCHULL, W. J. Human Heredity. Chicago, Ill.: The University of Chicago Press, 1954.
32. PENROSE, L. S. *In:* The Hazards to Man of Nuclear and Allied Radiations. London: Her Majesty's Stationary Office, 1956.
33. ———. A Note on the Prevalence of Genes for Deleterious Recessive Traits in Man. Ann. Human Genet., **21**:222–24, 1957.
34. PHILIP, U., and SORSBY, A. Quoted in Falls and Neel, 1951.
35. REED, S. C. A Test for Heterozygous Deleterious Recessives. J. Hered., **45**:17–18, 1954.
36. REED, T. E., and NEEL, J. V. A Genetic Study of Multiple Polyposis of the Colon (with an Appendix Deriving a Method of Estimating Relative Fitness). Am. J. Human Genet., **7**:236–63, 1955.
37. REESE, A. B. Frequency of Retinoblastoma in the Progeny of Parents Who Have Survived the Disease. Arch. Ophth., **52**:815–18, 1954.
38. SLATIS, H. A Method of Estimating the Frequency of Abnormal Recessive Genes in Man. Am. J. Human Genet., **6**:412–17, 1954.
39. SNYDER, L. S., and DAVID, P. Penetrance and Expression. *In:* Clinical Genetics. St. Louis, Mo.: C. V. Mosby Co., 1953.
40. STERN, C. The Hardy-Weinberg Law. Science, **97**:137–38, 1943.
41. ———. Principles of Human Genetics. San Francisco, Cal.: W. H. Freeman & Co., 1949.
42. TUCKER, D. P.; STEINBERG, A. G.; and COGAN, D. G. Frequency of Genetic Transmission of Sporadic Retinoblastoma. Arch. Ophth., **57**:532–35, 1957.
43. VOGEL, F. Über genetik und mutationsrate des retinoblastoms. Ztschr. menschl. Vererb., **34**:308–36, 1954.
44. ———. Neue untersuchungen zur genetik des rentinoblastoms (glioma retinae). *Ibid.*, **34**:205–36, 1957.
45. WEINBERG, W. Mathematische grundlagen der probandenmethode Ztschr. Indukt. Abstamm. Vererb., **48**:179–228, 1927.
46. WINTERS, R. W.; GRAHAM, J. B.; WILLIAMS, T. F.; MCFALLS, V. W.; and BURNETT, C. H. A Genetic Study of Familial Hypophosphatemia and Vitamin D Resistant Rickets. Trans. Assoc. Am. Physicians, **70**: 234–42, 1957.
47. WOLFF, J. A., and BERTUCCIO, M. A Sex-linked Genetic Syndrome in a Negro Family Manifested by Thrombocytopenia, Eczema, Bloody Diarrhea, Recurrent Infection, Anemia, and Epistaxis. J. Dis. Child., **93**:74, 1957.
48. WRIGHT, S. Coefficients of Inbreeding and Relationship. Am. Nat., **56**:330–38, 1922.

Importance of Genetics of Viruses in Medical Research

HILARY KOPROWSKI, M.D.
The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

There was a young lady named Rood,
Who was such an absolute prude,
That she pulled down the blind
When changing her mind,
Lest a curious eye should intrude.
—*The World's Best Limericks* (Mount Vernon, N.Y.: Peter Pauper Press)

Milnes était un garçon d'esprit qui faisait, et ce qui est plus rare, qui disait beaucoup de bêtises.
—ALEXIS DE TOCQUEVILLE, *Souvenirs*

When Doctor Lederberg flattered my ego by assigning to me the noble task of discussing the importance of virus genetics in medical research, the situation smacked of the one described by Sir Osbert Sitwell in his *Noble Essences*. In this anecdote, a robust old country neighbor was heard during a severe thunderstorm to address his faithful and aging servant: "Alec, you damn fool, don't stand about there doing nothing. Climb up the lightning conductor, can't you, and see if it's working." Thus "Climbing up the Lighting Conductor" becomes the first motto of this presentation.

The second has been provided by one of our "missile" generals testifying before a congressional committee during the post-Sputnik and pre-Explorer era. This general, when asked how our modern weapons compared with those of the Russians, replied: "We must catch up with them, but of course we are ahead of them." This is quite apt to the position of mammalian viruses *vis-à-vis* the genetics of phages. We are being exhorted to catch up with the phage geneticists; we have been subjected to merciless drubbing for the past decade because of the inexact methods we employ, because we show poor scientific judgment, and so on and so forth (and how true), and yet we are ahead of them because, while the phage geneticists were still playing around with phage-shockates that perhaps are pure DNA (though it is still doubtful) but which invade the bacteria only when the wall is removed, we are ahead of them because we discovered a mammalian virus RNA which penetrates intact cells.

The third motto is that of Marcus Annaeus Lucanus: "Metiri sua regna decet, vires-que fateri." Since all of you are familiar with Latin, you no doubt are sure that "vires" refers to viruses. Nothing could be further removed from the truth—and yet curiously enough this motto, too, is quite pertinent to our subject, for it means:

It Is Fitting To Measure His Own Powers, and To Admit His Strength.

. . . What such haste
Is yours, as that it cannot wait
fit times. . . .
—JOHN FORD, " 'Tis a Pity She's a Whore," in *Five Plays* (New York: Hill & Wang, Inc., 1957)

REMEMBRANCE OF THINGS PAST

Froissart's observation that "the most profitable thing in the world for the understanding of human life is history" can also be applied to the events which have led to the present-day concept of virus stability and virus mutation.

In what now seems like the historical past, the "subconscious" mind was appar-

ently groping toward virus genetics but had concentrated its attention on the one of the then easily discernible characters of a virus —its pathogenicity for the human species. All the early attempts at controlled manipulation of a virus had one objective in mind: to modify its disease-producing characteristics but at the same time to retain its immunizing power. This *leitmotiv* is encountered, with numerous variations, throughout the successive studies of viruses. Even today many of the detailed analyses of genetic properties were impelled by the desire to find a virus with definable characters which would serve to protect man or animal against disease.

Paradoxically, a variant of an otherwise highly virulent virus with the longest history has left for posterity very little to lean on. There is no doubt that Jenner used cowpox for human vaccination in his original investigations, but subsequent maintenance of the virus by man-to-man passages led to its contamination with smallpox (12, 35). Thus, although we can determine the character of the vaccinia virus employed for prophylactic measures, we are unable to define the origin of the virus. Repeated passages of cowpox in laboratory animals lead to a strain with vaccinia properties (3), but the occurrence of natural variants (13) would suggest that vaccinia may have arisen as a stable mutant in strains of cowpox.

The first cognitive attempt at manipulation of a virus, to obtain variation of its character, was done by Pasteur. Through passage in rabbits, the character of the wild type, or the so-called street virus of rabies, had undergone considerable change. Further variation in qualities was observed after passage in the developing chick embryo (32). The characteristics of the street virus and its three descendants (Table 1) range from complete absence of virulence of the HEP Flury strain, for any adult mammal, to full virulence of the street virus for any warm-blooded animal. The latter character is associated with the capacity of the virus to multiply *in vivo* in the salivary gland tissue. It should be mentioned at this point that rabies virus, because of its characteristics, is singularly unfitted for genetic study in which pure lines originating from a single virus particle must be employed.

TABLE 1

CHARACTERS OF FOUR VARIANTS OF RABIES VIRUS

RABIES STRAIN	INCUBATION PERIOD	PRESENCE SALIVARY GLAND	VIRULENCE							
			Rabbit		Mouse		Dog		Cow	
			i.c.	e.n.	i.c.	e.n.	i.c.	e.n.	i.c.	e.n.
Street	Long, variable	+++	+++	+++	+++	++	+++	+++	+++	+++
Fixed	Short	0	+++	+	+++	++	+++	+	+++	++
Flury LEP	Short	0	++	0	+++	±	±	0	+	+
Flury HEP	Short	0	0	0	0*	0	0	0	0	0

i.c. = Intracerebral inoculation.
e.n. = Extraneural inoculation.
0 to +++ = Nil to very high.

* Virulent for newborn mice only.
NOTE: All fixed strains, and many of the street strains, grow in chick embryo.

Another historical milestone was the emergence of the 17D strain of yellow fever virus (46) after passage of a virulent strain in mouse brain and in chick embryo tissue cultures. Continuous cultivation of the 17D strain, however, brought about one other, this time highly undesirable, character—loss of the immunizing property. While examining the data presented in Table 2, which by no means should be construed as being an exhaustive study of all the properties of the yellow fever strain, one is amazed at the paucity of defined markers for a virus like the 17D strain, which has been used, and is being used, for immunization of millions of people against yellow fever.

There are other "historic" examples of more or less stable variants of viruses produced by laboratory manipulation. This type of research is motivated to a great ex-

tent by the desire to decrease virulence of a virus for an economically important host, and is characterized chiefly by its unpredictability. No attempts were made, and no attempts could have been made because of lack of suitable techniques, to work with anything other than heterogenous viral populations. This led to selection of properties usually unreproducible for the second time. A notable exception was the study of the vaccinia virus (13).

The variations in influenza viruses are by now also history. However, in this case the less my arguments would fail without bringing forth the "flu and its variants."

There is an active tedium just as
there is a passive one.
—HELMUT KUHN, *Encounter with Nothingness*
(London: Methuen & Co., 1951)

MARKERS—MARKERS—MARKERS

Viruses as antigenic entities evoke serologic reactions which are characteristic of their immunological identity. Performance of the serologic tests is quite cumbersome;

TABLE 2

SOME DISTINGUISHABLE MARKERS OF THE ORIGINAL AND TWO DESCENDANT STRAINS OF YELLOW FEVER VIRUS

	Virulence			
	Man	Monkey		Immunization
Strain		i.c.	e.n.	of man
Asibi	++	+++	+++	+++
17D	0	±	0	+++
17DD High	0	?	0	± or 0

i.c. = Intracerebral inoculation.
e.n. = Extraneural inoculation.
0 to +++ = Nil to very high.

change from the human to the laboratory form (chick embryo cultivated) has been minutely studied and stands out as the first purposeful attempt at genetic analysis of inheritable characteristics in a mammalian virus (4, 6, 7). In brief, the influenza virus isolated from man (the O form, Table 3) can be cultivated after inoculation into the amniotic sac of fertile hens' eggs. In addition, this virus will display other properties, summarized in Table 3. In contrast, the virus which has been manipulated sufficiently to grow in the allantoic cavity (D form) displays entirely different characteristics (Table 3). If a fresh human isolate is maintained in cultures of human embryonic lung, it retains the property of the O form (37).

There are numerous reports on studies of influenza and other myxoviruses with descriptions of genetic markers and genetic analyses of the inheritance of demonstrable characteristics. Several reviews have been published recently; therefore, I shall abstain from dipping into the "influenza book," un-

TABLE 3

MARKERS OF THE TWO FORMS OF INFLUENZA*

	O Form	D Form
RBC Agglutination:		
Human cells	+++	+++
Guinea pig cells	+++	+++
Chicken cells	0	+++
Site of Infection:		
In chick embryo	Amnion	Allantois
Reaction with ovo-mucins	0	+++
Pathogenicity for man	++	0

* After Burnet (4).

and were this the only method available for the genetic study of viruses, progress in this field would be very slow indeed. It is not the purpose of this review to assemble all characteristic markers of many viruses, and only a few examples will be discussed which, because of their relative simplicity, could be used for investigation of genetic constancy of a given virus strain.

The ether and bile salt resistance permits classification of viruses into four unequal groups (Table 4) (2, 43, 47, 49): (*a*) Viruses inactivated by ether and desoxycholate form

the largest group—it comprises the myxoviruses (including mumps, influenza, Newcastle, etc.); Rous sarcoma and most of the encephalitides which belong to the serologically distinct group called Arbor A and B (8); (*b*) the pox viruses and human and mouse polio belong to the ether and desoxycholate resistant group; (*c*) psittacosis is inactivated by ether but not by DCA; and (*d*) rabies behaves in an opposite way.

TABLE 4

SUSCEPTIBILITY OF VIRUSES TO ETHER AND BILE-SALTS

DCA+ ET+	DCA− ET+	DCA− ET−	DCA+ ET−
Myxoviruses	Psittacosis	Pox viruses	Rabies
		Poliovirus	
Rous sarcoma		Mengo*	
		Mouse enceph.	
Rift Valley fever			
Eastern eq. enc.			
Western eq. enc.			
Louping ill			
Yellow fever			
St. Louis enc.			

DCA+ = inactivated by sodium desoxycholate.
DCA− = sodium desoxycholate resistant.
ET+ = inactivated by ethyl ether.
ET− = resistant to ethyl ether.

* = ether sensitivity not known.

See References 2, 43, 47, 49.

TABLE 5

CRITERIA OF CHARACTERS OF THREE POX VIRUSES

Pock appearance
Pock size
Viral content of pock
Virulence for mice
Infectivity for rabbits
Hemagglutinins
Heat resistance

A more sophisticated report on genetic markers has been presented by Fenner (20) for three pox viruses: vaccinia, cowpox, and rabbit pox. Eight different characters related to pock appearance, size, and infectivity, virulence for mice and rabbits, hemagglutinin production, and temperature susceptibility were studied (Table 5). The variability in the biological characters of the virus is best illustrated by the distribution of different markers among the 23 strains investigated (Table 6). Fenner divided his strains into two large groups: those producing small nodular lesions without necrosis, called the dermal strain, and those causing a large, flat lesion with extensive necrosis. Of the 23 studied only two strains of cowpox producing white pocks shared all other markers. Four strains differed only in relation to one character: pathogenicity for mice. In general, intracerebral virulence for mice and rabbits was associated with a red pock, and large hemorrhagic lesions were seen after intradermal inoculation of rabbits. Most of the strains, but not all, produced hemagglutinin, and most were heat-resistant.

To a great extent research on genetic markers of the poliomyelitis virus had the same motivation, that of decreasing its virulence, but the results of such studies have contributed greatly to the accumulation of basic data in the field of virus genetics.

Table 7 presents six different markers of polioviruses. The first refers to observations made by Dulbecco and Vogt (18) on the larger-sized plaques which appeared in the course of rapid passages of Brunhilde Type I virus on monolayer of renal epithelium from cynomolgus monkeys. This technique is em-

ployed widely in the study of quantitative aspects of virus growth, and the principal steps involved will be described briefly. A cell suspension is obtained either from organs (e.g., lung, kidney, etc., of adult animals) or from whole chick embryo or mouse embryo, and the cells are dispersed with the help of enzymatic digestion by trypsin or versene. A count of viable cells is made, and then the cells, resuspended in nutritive medium, are placed in petri dishes and exposed to virus infection. At given time intervals the virus is removed and the cellular monolayer is covered with an agar overlay, which is allowed to solidify; it is then incubated at 37° C. in the presence of CO_2, which helps to maintain the pH of the cultures at the desired levels.

The virus particles which had been adsorbed penetrate into the cells, multiply, and cause cellular necroses. These necrotic foci, called plaques, remain unstained areas among cells which take up neutral red stain as a sign of their viability. With this system, the multiplicity of infection (that is, the relationship between cells exposed to a given number of virus particles), the latent period (i.e., the time interval between adsorption and the release of first virus particle), and yield of virus (i.e., the number of virus particles released per cell) (39) are determinable variables. Genetic uniformity of the host cells is desirable, and today stable tissue culture strains can be obtained from single cells (39). This system enables isolation of virus progeny derived either from a single particle or from a single cell. It has found wide application in the study of genetics of numerous viruses and has permitted observation of the large plaque produced by a mutant of poliovirus referred to above.

Plaque size, as a characteristic in the poliovirus system, has been recently supplanted by a marker more readily determined, namely, the delayed appearance of plaques under agar overlay maintained in medium of low (0.55 gm/liter) bicarbonate content (see Table 7) (50). In an alkaline medium (2.75 gm/liter of bicarbonate), all strains of poliovirus tested show more or less equal efficiency of plating. In an acid medium, strains encountered in nature show similar plating efficiency, but the mutants, which show together with some other characteristics a decreased neuropathogenicity for rhesus monkeys, grow at a markedly delayed rate as compared with the rate in the alkaline medium; this character has been used effectively in the study of the rate of mutation of polioviruses (19) and will be discussed briefly later.

Strains with increased heat resistance have been reported for Type II (45) and

TABLE 6

DISTINGUISHING CHARACTERS OF 23 STRAINS OF POX VIRUSES*

LESION RABBIT SKIN	RATIO OF STRAINS SHOWING FOLLOWING PROPERTIES										
	Pocks						Virulence		HA	Heat resistance	
	White	Red	Small	Large	High Virus	Low Virus	Mice	Rabbit	Prod.	High	Low
Small nodule	12/13	1/13	3/13	1/13	10/13	3/13	3/13	3/13	9/13	11/13	2/13
Large lesion	1/10	9/10	2/10	8/10	8/10	2/10	7/10	6/10	7/10	10/10	

* After Fenner (20).
HA = Hemagglutinin production.

TABLE 7

GENETIC MARKERS OF POLIOMYELITIS VIRUSES USED IN THE STUDY OF MUTANTS

1. Plaque size on monolayer of monkey kidney
2. Plating efficiency under agar of low bicarbonate content
3. Heat resistance
4. Growth in tissue cultures maintained at 30° C.
5. Relation to presence of cystine in tissue culture medium
6. Inhibition by normal bovine serum

Type I (17) viruses. Another character of polioviruses, determined for strains representing the three types, is the capacity to grow in tissue culture cells maintained at 30° C., or even at 23° C. (15). The observation that the optimal cytopathogenic effect of polioviruses is dependent on the presence of cystine (Chart 1) in monkey kidney tissue cultures, led to the isolation of variants which do not require cystine for cytopathogenicity and which, furthermore, have their growth inhibited by high concentrations of cystine in the tissue culture medium (14). This is shown in Chart 2, which, in addition, serves as an illustration of plaques caused by single virus particles creating in the process of multiplication necrotic foci on monkey renal epithelium monolayer, as mentioned above.

Certain sera from otherwise normal cattle were found to neutralize polioviruses. This inhibiting effect, apparently unrelated to specific neutralization, permitted selection of strains which, in contrast to those encountered in nature, became resistant to inactivation by the bovine serum inhibitor (48).

As mentioned previously, to enumerate characters of different viruses is beyond the scope of this presentation. It should suffice to say that these markers permit us a deeper approach to the study of one of the most important and intriguing aspects of virus behavior, i.e., of mutation.

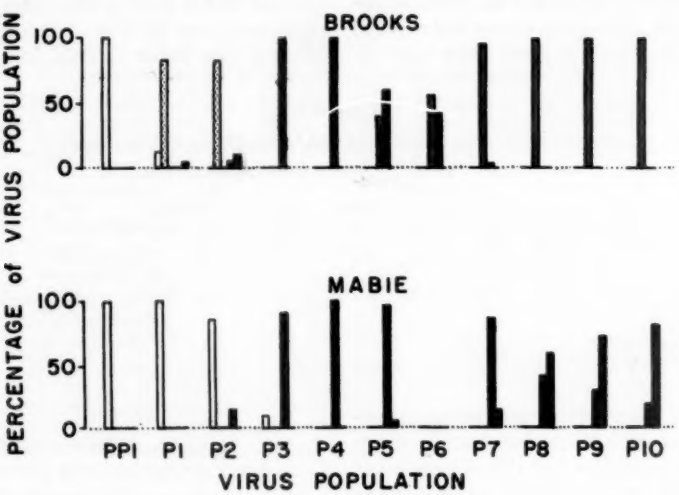


CHART 1

Virus Symbol	Response to Cystine: Requirement for	Response to Cystine: Inhibition by	Virus Symbol	Response to Cystine: Requirement for	Response to Cystine: Inhibition by
+cr	++	0	cr^{si}	0	+
cr^{p}	+	0	cr^{i}	0	++
cr	0	0			

After Dubes and Chapin (14).

Truth fails not, but her outward forms that bear
The longest date do melt like frosty rime

That in the morning whitened hill and plain
And is no more.
—WORDSWORTH, "Mutability," *The Weekend Book* (London: Nonsuch Press, 1955)

MUTATION, VARIATION AND ATTENUATION

Conforming to the pattern established in the preceding chapters, no attempt will be made to present an all-encompassing review of recent investigations on mutations in the field of animal viruses. Instead, examples of variation and attenuation of poliovirus will be given, since they represent a continuing challenge to investigators to correlate the readily determined characters of the virus with the elusive character of attenuation.

The early attempts to modify the disease-producing character of poliovirus followed the "historic" pattern, i.e., of adaptation to a hitherto seemingly unsusceptible host and then waiting for the grace of God to take the teeth out of the virus. This author was one of the early culprits in this scheme, and obtained strains of Type I and Type II which grew in brains of laboratory rodents (33, 34) and subsequently were tested for their relative avirulence in rhesus monkeys when injected directly into the brain. The rodent-adapted strains of poliovirus were fed to human subjects who supported the multiplication of the virus in the intestinal tract and developed antibodies, without any signs of illness whatsoever.

The advent of tissue culture techniques enabled more efficient and better controlled methods of study of virus variation. One of the methods, already of some historical value today, consisted of rapid passages of virulent virus population of high concentration in monkey kidney tissue culture tubes, or in bottles (40). The presumptive variants were then selected through limit-dilution passages and tested again in monkeys. Discovery of the plaque assay technique for poliovirus has permitted a more exact search for apathogenic strains of poliovirus. Progenies of single clones were obtained, studied for their neuropathogenicity, and the procedure of cloning was repeated until a clone with lesser pathogenicity was encountered. It involved the use of hundreds of rhesus and cynomolgus monkeys, and when the question of pathogenicity for intracerebrally injected monkeys was nearing solution, it was found that by the intraspinal route of inoculation virulent particles of the virus could still be detected.

Certain interesting facts were uncovered in the course of these investigations, e.g., that the virulence for the laboratory rodents had no relation to the neuropathogenicity for monkeys; that strains still pathogenic for monkeys were innocuous for chimpanzees injected either intracerebrally or intraspinally (41); that multiplication of virus in the human intestinal tract was unrelated to the action of the virus on monkey neurons; that strains isolated from healthy children varied widely in their pathogenicity for monkeys.

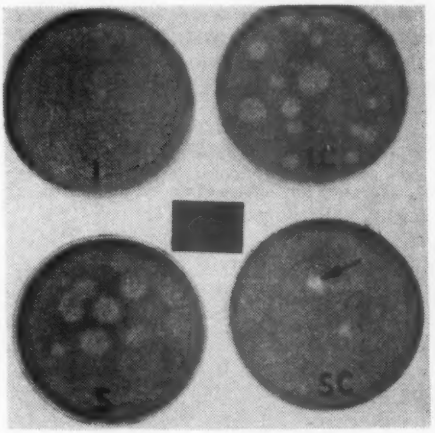


CHART 2.—Plaques formed by a cystine-requiring strain of poliovirus (Akron) and by a cystine-inhibited mutant of this strain.

1 = cystine-requiring virus in cystine-deficient medium.
1 C = cystine-requiring virus in medium with added cystine.
5 = cystine-inhibited mutant virus in cystine-deficient medium.
5 C = cystine-inhibited mutant virus in medium with added cystine.

After Dubes and Chapin (14).

Many of the strains, as mentioned above, have been tested in man and were found to multiply in the intestinal tract and to produce immunity. One notable exception was a strain with a rather interesting history of growth in the developing chick embryo (30). This Type II strain had a poor infectivity ratio for the human intestinal tract. Attempts were made to analyze the virus population of this strain and to determine whether the progenies were of uniform character. As shown in Chart 3, five clones were obtained from this strain after it was passed through the human intestinal tract. Four

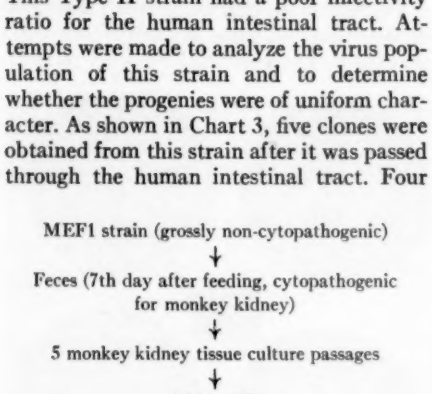

MEF1 strain (grossly non-cytopathogenic)
Feces (7th day after feeding, cytopathogenic for monkey kidney)
5 monkey kidney tissue culture passages
"Plaqued"
1* 2 3* 4 5
Mouse + CE + MK + HeLa +
Mouse + CE − MK + HeLa +
CE+ = multiplication in fertile hen's egg.
* fed to man—poorly antigenic.

CHART 3.—Jack. Infant fed chick embryo adapted

clones grew on monkey kidney, in mice, and in chick embryo; one failed to grow in chick embryo, which indicated a mixed population of the virus, at least in relation to one determined character. The uniform inability to infect the human intestinal tract persisted as characteristic of all progenies.

It was a paradox that this relatively crude, although interesting, wrestling with the problem of apathogenicity of a virus remained for a long time a phenomenon in itself, without any associated expressions of physiologic characteristics applicable in more exact and more quantitative determinations. The crudeness of the testing system, and the fear of reversion to virulence after passage through the human intestinal tract, prompted several investigators to devise methods which would elicit markers of definite character. Availability of these markers, summarized in Table 7, permitted a study of associations with apathogenicity. This can best be illustrated by citing Dulbecco's and Vogt's (19) correlation of the mutants, characterized by delayed growth in acid medium, with monkey virulence. The ratio of plaque-forming units per ml. of a given virus on a given day in acid medium to the maximal titer in alkaline medium, given the code eop-ac (efficiency of plating [under] acid agar), has been correlated with neuropathogenicity (19). Figures for eop-ac ratio of 2×10^{-1} to 9×10^{-2} were obtained for virulent virus designated as *d*+ "wild" variants. Of the ten relatively nonpathogenic strains, the eop-ac for nine varied from 1×10^{-4} to 4×10^{-6}, and the strains have been designated as "attenuated" *d* variants. Although this eop-ac ratio seems to be closely connected with the lack of neuropathogenicity, one attenuated strain showed wild-type eop-ac ratio.

Detailed analyses of the mild *d* lines disclosed (19) that these lines give rise to mutants similar to the wild-type virus. Some of these mutants called reverse mutants arose during growth of virus in a liquid medium and are seen as a few early-appearing plaques encountered on acid-agar on the fourth day of the inoculation of the mild *d* lines. On the other hand, some mutants may produce plaques on acid agar if the *d* lines are incubated for longer periods of time (6–7 days).

Plaques harvested from acid agar plates seeded with various mild *d* lines were then analyzed for the pattern of reverse mutability. The results shown by Dulbecco and Vogt (19) for two types of poliovirus are copied on Table 8. It may be observed that, in the case of Type I virus, the mild *d* mutant (LS-c-Reed 13 ab KP 2) reached an eop-ac ratio of 10^{-4} on the sixth day. This line reverted to a mutant with the same eop-ac as the wild *d*+ type of Mahoney virus. The Type III mild *d* line had a lower value

of 4×10^{-6} for eop-ac as compared with Type I virus. However, it reverted to the type having still lower eop-ac than the wild *d*+ Leon virus. The reversion of Type I virus to a wild mutant may have occurred in a single step; the reversion of Type III may need successive steps; the mutation frequency was found to be $2\text{–}3 \times 10^{-5}$ per particle per duplication. Paradoxically, in contrast to the tissue culture experiments, the "reversion" to lines more neuropathogenic for monkeys after one passage of the attenuated *d* lines through human intestinal tract occurs more frequently in Type III infection than in Type I (40, 41). This in itself may only indicate that attempts to compare a delicate quantitative genetic analysis, such as this, with a brutal blind inoculation of the same material into the spinal cord of a monkey—when pathways of infection, cycles of multiplication, etc., are entirely unknown—is of little scientific import. One may facetiously compare such a procedure with one which has never been performed and at which, no doubt, all phage-workers would throw up their hands in horror: for instance, inject bacteriophage into a cow and correlate the bovine infection (if any) with detailed genetic analysis of the phage particles forming the inoculum.

Since monkeys are of heterogenous stock, and animal virologists notoriously disregard genetics of the host, a fact which will be dealt with briefly later, one may present an additional argument against this type of test for attenuation, an argument which in the end may sound rather mildly absurd. It runs, or rather stumbles, somewhat like this: the single monkey which will become paralyzed out of ten, or hundreds, injected with the "attenuated" variant of poliovirus may have been genetically predisposed (his own genetics) to paralysis, and this fact should not have been interpreted as "selection of a reverse mutant."

Regardless of how absurd this argument may be, it is quite certain that virulence of a virus for a given species is a property of which the underlying mechanism is still improperly understood, and the genetic tools available today may not be sufficiently refined to investigate this phenomenon adequately.

TABLE 8

PATTERN OF REVERSE MUTABILITY OF VARIOUS *d* LINES

TYPE	VIRUS STRAIN	LINE	EOP-AC ON THE 4th Day	5th Day	6th Day
1	Mahoney	d^+ (wild type) (KPLO)	$2\text{–}5\times10^{-1}$		
		d mutant (LS-c-REED 13ab KP2)			10^{-4}
		1st reverse mutants (p11, p13)	$7\text{–}8\times10^{-3}$	$1\text{–}2\times10^{-2}$	
		2d reverse mutants (p15, p16)	$4\text{–}5\times10^{-2}$	1×10^{-1}	
3	Leon	d^+ (wild type) (KP3)	$1\text{–}2\times10^{-1}$		
		d mutant ($12a_1b$)			4×10^{-6}
		1st reverse mutants (p1, p2, p3)			$4\text{–}5\times10^{-4}$
		2d reverse mutants (p7, 8)			
		3d reverse mutants (p9, p12)	$3\times10^{-3}\text{–}5\times10^{-2}$		
		4th reverse mutants (p13, p17)	$3\times10^{-2}\text{–}2\times10^{-1}$		

After Dulbecco and Vogt (19).

> Being divided, set out from colour,
> Disjunct in mid darkness
> Grazeth the light, one moving
> by the other.
> —EZRA POUND, *Canto XXXVI*

RECOMBINATIONS

Virologists, even those with a background of genetics, are only human. They like to put two things together; and the temptation is even greater if they have so many different characters to play with, as is the case with markers of mammalian viruses. So two viruses were "put together" into the same host system. And the results? One may find an answer to this in an old limerick:

> There was an old man of the Nore,
> The same shape behind as before.
> They did not know where
> To offer a chair,
> So he had to sit down on the floor.

must remain stable when passaged on limited dilutions, or as progenies of single clones. Transfer of neurotropic properties from one strain of influenza A virus to another seemed to create a recombinant in the hands of Burnet (4). In the experiments done by Hirst and Gotlieb (28, 30, 31), hybridization of the influenza A strain led to the appearance of a virus with serological properties of both parental strains and an endowment with some other markers; this may have been a possible case of heterozygosis. The identity of hybrids, obtained in the latter studies, was not retained in subsequent passages. However, it should be pointed out that a mutant was obtained by

TABLE 9

DIFFERENTIATING MARKERS OF THE INFLUENZA A STRAINS MEL AND WSE

MEL	WSE
A Serology MEL	a Serology WS
B Heat-stable HA	b Heat-labile HA
C Not indicator on heating to 55° (ovomucin)	c Indicator on heating (ovomucin)
D Not indicator on heating (sheep salivary mucin)	d Indicator on heating (sheep mucin)
E Not pathogenic for chick embryo	e Hemorrhagic lesions on CA inoculation
F* Weak lesions in mice inoculated intranasally	f Highly pathogenic for mice intranasally

* In some of the other combinations used in our experiments the symbol F was used for complete absence of lesions in mice inoculated intranasally. When any confusion might arise we have indicated the weak mouse virulence of MEL with the symbol in brackets (F).
After Burnet (4).

Reluctantly, I have to return to the influenza virus, since the first studies on genetic interaction were with strains of influenza A (4). As shown in Table 9, two strains with different serological characters, and several other markers which could be detected *in vitro*, were used for hybridization. Another important difference was, for instance, the intracerebral virulence for mice of one (the NWS) of the strains. Various types of matings occurred; in some instances seemingly true recombinants were obtained. Under this term (see Table 10) is to be understood a hybrid with serological properties of one strain, i.e., neutralized by serum containing antibody, and one or preferably more markers of the other strain. Such a virus has to breed true on subsequent passages, that is, its markers

Burnet from a strain of influenza A highly virulent for mice. This mutant possessed serologic characters of WSE but had *no* virulence for mice. Originally it was thought to be stable on passages at limited dilutions. Therefore, it was surprising to find that the loss of virulence for mice was not a true phenomenon, since this seemingly stable mutant, on passages in allantoic cavity of fertile hens' eggs, "reverted" to full mouse virulence (5).

The experimental tools available today do not permit a detailed analysis of heterozygosis versus phenotypic mixing. However, double infection with two strains of poliovirus seems to fall into the last category (36). Mating of Types I and II produced virus neutralized by Type I and Type II specific sera. The double neutralizable virus "broke

off" into the parental strain immediately after the first passage in tissue culture. Phenotypic mixing has been observed also in the mating of two strains of Newcastle virus (27). In this case, however, heterozygosis may also have taken place, though again tools for detailed analysis are not yet available.

Returning to the definition of true recombinants, experiments reported by Wildy (51) for Herpes simplex virus may indicate creation of new strains through mating of parents with different markers.

One must express his admiration for the painstaking research carried out in the pock areas of different types. About 30 per cent of single-particle progenies showed markers indicating recombination, as shown in Table 12. Some of these new viruses retained stable characters on repeated passages.

Thus, putting "two things together" may result in all sorts of reactions: in the production of legitimate offspring or homozygotes with some characters of one parent and some from the other; production of short-lived bastards who may inherit the outer characteristics (coat) of one parent but which are lost promptly (phenotypic mixing); and, finally, there are the "in-between" which are difficult to analyze without recourse to Freud, and which fall into the category of heterozygotes.

TABLE 10

EXAMPLES OF RECOMBINANTS, HETEROZYGOTES, AND PHENOTYPIC MIXING

Phenomenon	Viruses for Recombination	Authors
Recombination	Influenza A (several strains)	Burnet
	Herpes (WC×HFEM)	Wildy
	Vaccinia (neuro and dermal)	Fenner
Phenotypic mixing (possible heterozygosis)	Influenza A (MEL and NWS)	Hirst and Gottlieb
	Polio (types 1 and 3)	Sprunt, Ledinko
	Newcastle virus	Granoff

TABLE 11

MARKERS OF TWO STRAINS OF VACCINIA USED IN RECOMBINATION EXPERIMENTS

		Virulence			
		Mouse	Rabbit		Heat
Type	Pock	I.C.	Intradermal	Hemagogl.	resistance
Neuro-vaccinia	Red	High	Large lesion	None	High
Dermal-vaccinia	Gray	Low	Small nodule	High	Low

After Fenner (21).

study of markers of pox viruses. There were many markers available to Fenner to choose parents for mating in the vaccinia virus group. Finally two strains were selected which were exact opposites in their biologic characters (Table 11), and a double infection of chorioallantoic membranes was accomplished. In the first experiments, chorioallantoic membrane was inoculated with large doses of two parent strains, and properties of the virus, if any, were assayed 12 hours later. Of the 95 clones (single-particle progenies) tested, four recombinants were found (Table 12). In another experimental set-up Fenner (21) injected chorioallantois with mixtures of parent strains which give rise to overlapping of

And he said to himself as he bolted the door,
"I will not wear a similar dress any more,
Any more, any more, any more, never more!"

—*The Complete Nonsense of Edward Lear* (London: Faber & Faber, 1947)

ARE GENETIC MARKERS OF INTACT VIRUS STILL NEEDED?

It has been suspected for some time that the nucleic acid in the nucleoprotein complexes, which we call viruses, plays the prominent role as transmitter of genetic in-

formation. To prove this point beyond any possibility of doubt, it became necessary to divest the virus of its protein dress and view it in its naked but still recognizable form. The act of undressing may be a pleasurable one or not, depending on the circumstances, but it is difficult to put one's self in the position of a virus in the process of shedding its clothing. During the past year, Colter *et al.* (10, 11) were able to undress three mammalian viruses by means of a bath in water-saturated phenol. Removal of phenol by ether extraction completed the undressing, and the virus stood in all its naked, infectious splendor. In Table 13 are shown examples of five viruses which were subjected to such undressing and which, when naked, consisted of ribonucleic acid deprived of its protein coat, but still infectious.

Since the technique of phenol extraction is today quite a simple laboratory procedure, it is possible that, while this review is being read, other viral agents have been similarly undressed. This, however, is not true of all viruses. Attempts have been made in various laboratories to isolate an infectious RNA from viruses which belong to the "genetically belabored" myxo-group, such as fowl plague and influenza. Thus far, for some unexplained reason, these viruses have not been amenable to the wiles of the "undressers."

TABLE 12

EXAMPLES OF THE STABLE RECOMBINANTS OBTAINED IN EXPERIMENTS WITH RABBITPOX AND DERMAL VACCINIA VIRUSES

Italics indicate the features in which the strain in question differs from the parent dermal vaccinia strain.

Strain	CAM	MV	RS	HA	HA
Rabbitpox parent	R	h	PC	*0*	h
Dermal vaccinia parent	W	l	N	h	l
R1-1	W	1	N	*0*	1
R1-3	W	*int.**	*int.*	h	1
R1-4	W	1	N	h	*h*
R2-3	W	*int.*	*int.*	h	*h*

* int. = intermediate between the behavior of the two parent strains.

CAM = pock appearance
MV = mouse virulence
R = hemorrhagic
W = opaque white
RS = intradermal inoculation of rabbits
HA = hemagglutinin production
h = high
l = low
HR = heat resistance
PC = purple center
N = red nodule

After Fenner (21).

TABLE 13

INFECTIOUS RNA OF MAMMALIAN VIRUSES

Virus	Authors
West Nile	Colter *et al.*
Mengo	Colter *et al.*
Polio	Colter *et al.* Alexander *et al.*
Eastern equine encephalitis	Wecker and Schäfer
Semliki forest	Chang

The infectivity of the five ribonucleic acids did not exceed 0.1 per cent of that of the viruses from which they were isolated (9). Properties of the naked RNA and the intact virus are summarized in Table 14. In contrast to the intact virus, the infectivity of RNA was abolished by treatment with ribonuclease and 6 hours of incubation at 37° C. Treatment with 1 M NaCl for 12–16 hours markedly decreased the infectivity of the intact virus, but it had little or no effect on the infectious RNA. As was to be expected, the intact virus particles sedimented more rapidly than the infectious component in the RNA preparation (9).

The action of serum requires more explanation. In experiments conducted by Schramm and Gierer (42) with plant viruses, immune serum which neutralized the intact

tobacco mosaic virus (TMV) had no effect on the infectious, naked RNA component, although both normal and immune serum showed a certain amount of inactivating capacity. Unfortunately, this was much more accentuated in the case of animal virus RNA, and, therefore, the elegant experiments showing lack of effect of immune serum in the absence of protein dress are as yet inconclusive.

Even if the fact is taken into consideration that some viruses are reluctant to shed their protein dress, and may remain so forever, the availability of several infectious ribonucleic acids isolated from different viral preparations may be of enormous importance for the genetics of animal viruses. The study of configuration of bases in the RNA preparation may already show differences between various viruses. This may be wishful scientific optimism, but it should be remembered that Ada and Perry (1) have shown that ribonucleic acid (in an impure preparation) of influenza A virus had a characteristically different pattern of bases from that of influenza B. The availability in the future of such chemically defined characters could make the other genetic markers of viruses discussed above look rather obsolete.

The availability of an infectious RNA may also help to elucidate, at least partially, the comparative importance of naked RNA *vis-à-vis* its nucleoprotein complex in the carrying out of biological activity. The question arises whether the nucleic acid is the sole genetic marker, the protein part acting only as a "public relations department" (38). To discuss the problem intelligently one has to digress for a moment from the fauna of animal viruses and invade again the flora of plant viruses. The naked RNA of tobacco mosaic virus is infectious. However, when mixed with the inactive protein component of TMV the infectivity is increased. This, and other arguments, including that of electron micrograph preparations, would seem to indicate that, through recombination of RNA and protein, a reconstituted virus was formed (24). Thus, at least partial evidence was obtained that the two components of TMV virus recombined in a specific manner. But, which component was the master of biological activity?

In some rather complicated experiments Fraenkel-Conrat and Singer (22, 23) have reconstituted viruses through recombination of RNA and inactive viral protein obtained from two or more different strains of TMV. These reconstituted nucleoproteins were tested for infectivity in plants, and the resulting progenies were chemically and serologically analyzed. The results indicated that the protein of the progeny resembled, or was identical with, that of the protein from which the infectious RNA had been obtained and seemingly was unrelated to the protein which was used for the reconstitution of the inoculum (22, 23). Although an attempt was made to prove that in certain instances the protein dress may influence the character of nucleic acid (52), in essence, we may accept the possibility that the RNA of the TMV virus is the master of genetic activity. The public relations role of protein has also been proved through the observation that immune serums were specific for protein components (23).

TABLE 14

COMPARISON OF CHARACTERISTICS OF INTACT VIRUS AND VIRUS RNA

Test	Intact virus	Virus RNA
Ribonuclease	No effect	Inactivation
6 hours at 37 C.	No effect	Inactivation
1 M NaCl	Decreased infectivity	No effect
Sedimentation velocity	Rapid	Slow
Immune serum	Neutralization	?*

* See text for discussion on "inactivation" by normal serum.

In the case of animal virus RNA it is not possible as yet to conduct the same sort of experiments as were done with TMV because of impurity of animal virus preparations. However, the role of RNA could be ascertained if exposure of the test system—hitherto found resistant to intact virus infections—were attempted. Would such a system be susceptible to the naked RNA, in contrast to the intact virus? A parallel perhaps may be drawn with the studies of bacteriophage DNA (44, 52).

The T2 phage disrupted by osmotic shock, and containing infectious DNA, cannot infect bacteria which are susceptible to the intact phage. However, the shockates will infect protoplast not only of susceptible strains but also of some resistant strains (44). In the introduction mention had been made that in this one aspect the animal virologists have advanced beyond the phage-geneticists; their infectious RNA preparations consist of protein-free nucleic acid, whereas the phage DNA may still be protein-bound (25) but probably not for very long in terms of chronology of scientific observations.

TABLE 15

EXTREMES OF HOST SPECIFICITY

Virus	Host Range
Rabies	All homeothermal animals
Influenza	Man, ferret, mouse, chick embryo
Adeno	Man and tissue culture
AKR mouse leukemia	Z subline of C3H strain of mice*

* The susceptibility of the AKR strain cannot be easily verified because of the incidence of spontaneous leukemia.

In the forthcoming (I hope) studies on the role of RNA as determinant of genetic constitution of the particles, recombination experiments between two different RNA may be even more exact and more exciting. Many other discoveries may be made by using infectious mammalian RNA as an experimental source, and a new era of animal virus genetics may be in the offing. The naked virus may yet become the dressed, in any style it may desire.

There lived an old man in the Kingdom of Tess,
Who invented a purely original dress;
And when it was perfectly made and complete
He opened the door and walked in the street . . .

The last class of illusions are those which cannot be discovered within one person's experience, except through the discovery of discrepancies with the experiences of others.—BERTRAND RUSSELL, *Mysticism and Logic* (Doubleday, W.V., 1951)

IS THE HOST NECESSARY?

It would be unwise to leave the audience with illusions of starry-eyed optimism, and it should be brought back to earth with a question: After all that has been said, is the host still necessary? Obviously yes, not only to provide the viruses with rich soil for their propagation, mutation, and any other tricks of their trade to enable their survival, but also to furnish the workers in the field a source for receiving grants for their studies. If the host is necessary, should it then be disregarded? How much can the host contribute in its intact (and not in tissue culture) form to our knowledge of virus genetics? The answer, again, is quite a lot.

The last table (number 15) summarizes the extremes of host range for viruses. Universal susceptibility to rabies virus may be sharply contrasted with the extreme specific susceptibility of inbred sublines of a highly inbred strain of mice to a leukemia virus. Despite the wide spectrum of animals susceptible to rabies infection, why do tissue culture explants stubbornly resist the infection with the same virus, in marked contrast to the adeno-viruses which grow with equal ease in man and in his tissues *ex corpora*? Is the disregard for the genetic origin of the host justifiable? Are kidney cell explants obtained from different monkeys at various time intervals similar in their susceptibility to virus infection, though obtained from a heterozygous breed? Should "adaptation" of a virus to a Swiss mouse lead to shouts of joy, or rather to expression of sorrow that a genetically inbred strain of mice had not

been retained as host in lieu of a heterozygous stock. What is adaptation, virulence, attenuation?

Answers to these questions are either unavailable or within the realm of delightfully speculative hypotheses, indicating that, wise as we may seem to be, there still remains much to be learned.

The art of quotation requires more delicacy in the practice than those conceive who can see nothing more in a quotation than an extract.—DISRAELI

ENVOI—SCIENCE AND MAGIC

Sir James G. Frazer has this to say in *The Golden Bough* (26):

But while science has this much in common with magic that both rest on a faith in order as the underlying principle of all things, readers of this work will hardly need to be reminded that the order presupposed by magic differs widely from that which forms the basis of science.

And yet later on he qualified this:

We must remember that at bottom the generalizations of science, or, in common parlance, the laws of nature are merely hypotheses devised to explain that ever-shifting phantasmagoria of thought which we dignify with the high-sounding names of the world and the Universe. In the last analysis magic, religion, and science are *nothing but theories of thought*. (Italics are mine.)

It must by now be quite clear that "faith in order as the underlying principle" of genetics of animal viruses is based as much on "theories of thought" as on "patient, exact observation of the phenomena themselves." If this faith is somewhat wobbly at times, it is because of the existing confusion which prevents us from seeing "order as the underlying principle" in the classification of the hosts to the viruses—that is, animals.

Robert Graves (29) quotes a story which he calls "ancient." An old lady traveling by train from London to Edinburgh was taking a pet tortoise in a basket and wanted to know whether she should buy a dog-ticket for it, as one is required to do in England for cats because cats officially count as dogs. "No," said the ticket inspector, "no mum. Cats is dogs, and rabbits is dogs, and dogs is dogs; and squirrels in cages is parrots, but this here turkle is a hinsect. We won't charge you nothing, mum!"

REFERENCES

1. ADA, G. L., and PERRY, B. Specific Differences in Nucleic Acids from A and B Strains of Influenza Virus. Nature, **175**:854–55, 1955.
2. ANDREWES, C. H., and HORSTMANN, D. M. The Susceptibility of Viruses to Ethyl Ether. J. Gen. Microbiol., **3**:290–97, 1949.
3. BLANK, H., and RAKE, G. Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man. Boston: Little, Brown & Co., 1955.
4. BURNET, F. M. Genetics of Animal Viruses. Handbuch der Virusforschung, **4**:1–26. Wien: Springer-Verlag, 1958.
5. ———. Genetics of Influenza Virus. Australian J. Sc., **20**:74–75, 1957.
6. BURNET, F. M., and BULL, D. R. Changes in Influenza Virus Associated with Adaptation to Passage in Chick Embryos. Australian J. Exper. Biol. Med. Sc., **21**:55–69. 1943.
7. BURNET, F. M., and STONE, J. D. Further Studies on O-D Changes in Influenza Virus A. Australian J. Exper. Biol. Med. Sc., **23**:151–60, 1945.
8. CASALS, J. Viruses: The Versatile Parasites. I. The Arthropod-Borne Group of Animal Viruses. Trans. New York Acad. Sc., Ser. 2, **19**:219–35, 1957.
9. COLTER, J. S. Nucleic Acid as the Carrier of Viral Activity. Progress M. Virol., **1**:1–35, 1958.
10. COLTER, J. S.; BIRD, H. H.; and BROWN, R. A. Infectivity of Ribonucleic Acid from Ehrlich Ascites Tumor Cells Infected with Mengo Encephalitis. Nature, **179**:859–60, 1957.
11. COLTER, J. S.; BIRD, H. H.; MOYER, A. W.; and BROWN, R. A. Infectivity of Ribonucleic Acid Isolated from Virus-Infected Tissues. Virology, **4**:522–32, 1957.
12. DOWNIE, A. W. Jenner's Cowpox Inoculation (Jenner Memorial Lecture). Brit. M. J., **2**:251–56, 1951.

13. DOWNIE, A. W., and HADDOCK, D. W. Variant of Cowpox Virus; Preliminary Communication. Lancet, **1**:1049–50, 1952.
14. DUBES, G. R., and CHAPIN, M. Poliovirus Mutants with Altered Responses to Cystine. J. Gen. Microbiol., **18**:320–29, 1958.
15. DUBES, G. R., and WENNER, H. A. Virulence of Polioviruses in Relation to Variant Characteristics Distinguishable on Cells *in Vitro*. Virology, **4**:275–96, 1957.
16. DULBECCO, R. Interactions of Viruses and Animal Cells; Study of Facts and Interpretations. Physiol. Rev., **35**:301–35, 1955.
17. ———. Quantitative Aspects of Virus Growth in Cultivated Animal Cells. *In:* Ciba Symposium on The Nature of Viruses, p. 147, 1956.
18. DULBECCO, R., and VOGT, M. Biological Properties of Poliomyelitis Viruses as Studied by the Plaque Technique. Ann. New York Acad. Sc., **61**:790–800, 1955.
19. ———. Study of the Mutability of *d* Lines of Polioviruses. Virology, **5**:220–35, 1958.
20. FENNER, F. The Biological Characters of Several Strains of Vaccinia, Cowpox and Rabbit-Pox Viruses. Virology, **5**:502–29, 1958.
21. ———. Genetic Studies with Vaccinia Virus. Australian J., Sc., **20**:75–76, 1957.
22. FRAENKEL-CONRAT, H. The Role of the Nucleic Acid in the Reconstitution of Active Tobacco Mosaic Virus. J. Am. Chem. Soc., **78**:882–83, 1956.
23. FRAENKEL-CONRAT, H., and SINGER, B. Virus Reconstitution. II. Combination of Protein and Nucleic Acid from Different Strains. Biochim. et Biophys. Acta, **24**: 540–48, 1957.
24. FRAENKEL-CONRAT, H., and WILLIAMS, R. C. Reconstitution of Active Tobacco Mosaic Virus from Its Inactive Protein and Nucleic Acid Components. Proc. Nat. Acad. Sc., **41**:690–98, 1955.
25 FRASER D.; MAHLER, H. R.; SHUG, A. L.; and THOMAS, C. A., JR. The Infection of Sub-Cellular *Escherichia coli*, Strain B, with a DNA Preparation from T2 Bacteriophage. Proc. Nat. Acad. Sc., **43**:939–47, 1957.
26. FRAZER, SIR J. G. The Golden Bough. New York: The MacMillan Co., 1958.
27. GRANOFF, A. (to be published).
28. GOTLIEB, T., and HIRST, G. K. Experimental Production of Combination Forms of Virus; Formation of Doubly Antigenic Particles from Influenza A and B Virus and Study of Ability of Individual Particles of X Virus To Yield Two Separate Strains. J. Exper. Med., **99**:307–20, 1954.
29. GRAVES, R. Five Pens in Hand. New York: Doubleday & Co., 1958.
30. HIRST, G. K., and GOTLIEB, T. Experimental Production of Combination Forms of Virus; Study of Serial Passage in Allantoic Sac of Agents That Combine Antigens of Two Distinct Influenza Strains. J. Exper. Med., **98**:53–70, 1953.
31. ———. The Experimental Production of Combination Forms of Virus. V. Alterations in the Virulence of Neurotropic Influenza Virus as a Result of Mixed Infection. Virology, **1**:221–35, 1955.
32. KOPROWSKI, H. Biological Modification of Rabies Virus as a Result of Its Adaptation to Chicks and Developing Chick Embryos. Bull. World Health Organ., **10**:709–24, 1954.
33. KOPROWSKI, H.; JERVIS, G. A.; and NORTON, T. W. Immune Responses in Human Volunteers upon Oral Administration of a Rodent-Adapted Strain of Poliomyelitis Virus. Am. J. Hyg., **55**:108–26, 1952.
34. KOPROWSKI, H.; NORTON, T. W.; JERVIS, G. A.; NELSON, T. L.; CHADWICK, D. L.; NELSEN, D. J.; and MEYER, K. F. Clinical Investigations on Attenuated Strains of Poliomyelitis Virus. Use as a Method of Immunization of Children with Living Virus. J.A.M.A., **160**:954–66, 1956.
35. LEDINGHAM, J. C. G. Comparative Study of Clinically Allied Viruses: Some Unsolved Problems of Edward Jenner: President's Address. Proc. Roy. Soc. Med., **29**:73–82, 1935.
36. LEDINKO, N. (to be published).
37. MOGABGAB, W. J.; GREEN, I. J.; and DIERKHISING, O. C. Primary Isolation and Propagation of Influenza Virus in Cultures of Human Embryonic Renal Tissue. Science, **120**:320–21, 1954.
38. MUDD, S. (personal communication).
39. PUCK, T. T., and MARCUS, P. I. A Rapid Method for Viable Cell Titration and Clone Production with HeLa Cells in Tissue Culture. Proc. Nat. Acad. Sc., **41**:432–37, 1955.
40. SABIN, A. B. Present Status of Attenuated Live-Virus Poliomyelitis Vaccine. J.A.M.A., **162**:1589–96, 1956.
41. ———. Properties of Attenuated Polioviruses and Their Behavior in Human Beings. *In:* Cellular Biology, Nucleic Acids

and Viruses, Special Publ. New York Acad. Sci., **5**:113–27, 1957.

42. SCHRAMM, G., and GIERER, A. Investigations on the Ribonucleic Acid of Tobacco Mosaic Virus. *In:* Cellular Biology, Nucleic Acids and Viruses, Special Publ. New York Acad. Sc., **5**:229–34, 1957.
43. SMITH, W. Action of Bile Salts on Viruses. J. Path. & Bact., **48**:557–71, 1939.
44. SPIZIZEN, J. Infection of Protoplasts by Disrupted T2 Virus. Proc. Nat. Acad. Sc., **43**:694–701, 1957.
45. STANLEY, N. F.; DORMAN, D. H.; PONSFORD, J.; and LARKIN, M. Isolation of a Heat-Resistant Variant of Polio-Virus. Nature, **178**:413–14, 1956.
46. STRODE, G. K. (ed.). Yellow Fever. New York: McGraw-Hill Book Co., 1951.
47. SULKIN, S. F., and ZARAFONETIS, C. Influence of Anesthesia on Experimental Neurotropic Virus Infections; *in vitro* Studies with Viruses of Western and Eastern Equine Encephalomyelitis, St. Louis Encephalitis, Poliomyelitis (Lansing) and Rabies. J. Exper. Med., **85**:559–69, 1947.
48. TAKEMORI, N.; NOMURA, S.; NAKANO, M.; MORIOKA, Y.; HENMI, M.; and KITAOKA, M. Mutation of Polioviruses to Resistance to Neutralizing Substances in Normal Bovine Sera. Virology, **5**:30–55, 1958.
49. THEILER, M. Action of Sodium Desoxycholate on Arthropod-Borne Viruses. Proc. Soc. Exper. Biol. & Med., **96**:380–82, 1957.
50. VOGT, M.; DULBECCO, R.; and WENNER, H. A. Mutants of Poliomyelitis Viruses with Reduced Efficiency of Plating in Acid Medium and Reduced Neuropathogenicity. Virology, **4**:141–55, 1957.
51. WILDY, P. Recombination with Herpes Simplex Virus. J. Gen. Microbiol., **13**:346–60, 1955.
52. WONG, T. Y., and COMMONER, B. The Formation of Infectious Nucleoprotein from Tobacco Mosaic Virus Protein and Tobacco Leaf DNA. Proc. Nat. Acad. Sc., **42**:831–41, 1956.

Discussion*

GEORGE K. HIRST
Public Health Research Inst., New York, N.Y.

The impact of genetics on the virus field, both bacterial and human, can best be appreciated if one tries to recall the confused state of knowledge concerning the nature of viruses as recently as 10 years ago. This confusion was rapidly resolved after Hershey and his co-workers demonstrated that viruses were organisms with a genetic apparatus very similar to that of higher forms. The demonstration that bacterial viruses had a chromosome made of DNA and bearing genes in linear array, however, did much more than unite viruses with other biological species. Through the study of bacterial viruses by genetic methods, it has been possible to get an increasingly clear picture of how viruses multiply and reproduce themselves within the host cell. We now have clues about the evolutionary relationship of the virus and the host cell. In addition, bacterial virology is making contributions to classical genetics and has been especially valuable in extending the biochemical attack on genetic and biosynthetic problems. The relationships between bacteria and their viruses are such that it is now impossible to study either virus or host cell without an intimate knowledge of the other. One of the effects of the study of genetics of bacteriophage has been the stimulation of similar studies in the animal virus field.

Animal viruses may be divided into two large groups based on the type of nucleic acid which they contain. Some are made up principally of DNA, and some contain RNA. So far, no virus contains both. Both kinds of virus give rise to mutations, and, with the use of these as markers, recombination experiments can be carried out. Among the DNA viruses, a few preliminary studies have shown that genetic recombination can take place between different strains of vaccinia virus and of herpes simplex virus. Among the RNA-containing viruses, influenza, Newcastle disease virus, and human poliomyelitis virus are the ones which have been principally studied. Thus far, recombination has been demonstrated only with influenza virus. Most of the genetic work with animal viruses has been done with the relatively clumsy tools which have been available for some time. No definitive studies have been done with any animal virus by the modern quantitative virological techniques of plaque formation and infection of single cells. It is not known, for example, for any animal virus, whether the genes for different characters are carried in a linear order. The problems of greatest interest lie in the future.

An example of the type of information which is already existent in studies of influenza and polioviruses concerns phenotypic mixing. When the distribution of genetic markers is studied following the infection of a cell with two different distinguishable virus strains, the progeny show certain changes in the phenotype, either in type specificity or in the nature of the hemagglutinin, and this alteration can be demonstrated to be non-inheritable in character. A mixed infection with type A and type B influenza viruses yields particles that have both A and B antigens on the surface of such particles. The situation seems to be that the genetic material, presumably the nucleic

* Following paper by Dr. Koprowski.

acid, from the two virus strains multiplies independently and specifically within the same host cell but that the coating of this material by virus-induced protein or other substance is a non-specific process and the nucleic acid from one strain may be coated with material characteristic of another strain. This is called phenotypic mixing and has been known for a long time with bacterial viruses.

With animal viruses, especially those of the myxo group, there is now independent evidence based on morphological findings which is consistent with this interpretation of phenotypic mixing (Breitenfeld and Schäfer, *Virology*, 4:328–45, 1957). When fowl plague virus, which is closely related to influenza A, infects an isolated cell (such as a chick fibroblast), studies with fluorescent antibodies reveal that an early consequence of this infection is the appearance in the nucleus of a nucleic acid or nucleoprotein which is characteristic of the virus and which does not appear initially in the cytoplasm. This nucleic acid or nucleoprotein cannot be detected by the same reagents in the intact virus particle, so it presumably is in some naked or uncovered state in the cell nucleus.

At a later stage of infection, the presence of coating material can be detected in the cytoplasm, and this coating material which appears diffusely throughout the entire cytoplasmic portion of the cell does not occur in the nucleus. At a still later stage, the demonstration of finished, coated particles can be seen in the cell membrane of the host cell by electron microscopy. Careful study of the infected cell shows that this coating takes place only in the cell wall. From this sort of evidence, it becomes readily understandable how coating occurs in a non-specific fashion.

In attempting to assess the importance of genetic studies on animal viruses, stress should be laid on their possible importance in those conditions where there is an intimate, long, and at present obscure relationship between the virus and the host cell. Such conditions are found pre-eminently in the relationship between tumor-inducing viruses and their host cells and in latent virus conditions. Alternatively, the genetics of mammalian viruses will probably be of little consequence in combatting infectious diseases where the virus has a purely cytopathogenic effect. The virus etiology of cancer is receiving increasing attention and offers possibilities of studying the mechanism of cancer inception in normal cells. It is not inconceivable that, with the continued expansion of the field of virology and the spread of the revolutionary techniques which have recently been introduced (especially in cell culture), we will witness a parallel development in the study of animal viruses and the study of somatic host cells in which each will complement the other.

Bacterial Genetics and Infectious Disease

B. A. D. STOCKER

Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1., England

Bacteriologists from Pasteur onward have been much concerned with "bacterial variation" and "dissociation," i.e., with problems of the genetics of bacteria, and with their relation to pathogenicity. In recent years the use of bacteria as experimental material for genetical research has led to discoveries of great importance to genetics and biology generally—e.g., to the identification of deoxyribonucleic acid as the material basis of the gene. The application to bacteriology of concepts developed in the study of the genetics of higher organisms has greatly increased our understanding of inheritance in bacteria. We now appreciate that in bacteria, as in higher organisms, it is essential to distinguish the phenotype, i.e., the actual observable characters of the individual organism, and the genotype, i.e., the sum of all the heritable characters, either actually expressed or capable of expression in a suitable environment; and that the phenotype of a bacterium is determined both by its genotype, or hereditary constitution, and by the environment in which it grows (in some cases the environment of the immediately preceding generations also has some influence). Bacteria divide by binary fission, and the genotype of the two daughter cells, and of their descendants, is identical with that of the parent bacterium, except when a change is caused either by *genetic recombination* or by *mutation*. These terms are here used in a wide sense—"recombination" to cover all cases in which a bacterium interacts with another organism (bacterium or bacteriophage) and in consequence produces progeny having an altered hereditary constitution; and "mutation" to describe all changes in hereditary constitution in which no second organism is involved.

In bacteria a great variety of characters are subject to change by mutation, different mutations occurring at characteristic rates, from as high as one in a hundred to as low as one in ten thousand million per bacterium per generation; the rate may be increased by mutagenic treatments, e.g., ultraviolet irradiation, or decreased by other treatments. In *Escherichia coli*, a species which "mates," genetical analysis has shown that many different mutants behave as if their mutant character resulted from a change at a localized spot in a linearly arranged group of genes (linkage map). In other genera, e.g., Pneumococcus, in which DNA transformations can be effected, it is found that a mutation in a bacterium is associated with a corresponding change in the transforming properties of the DNA extractable from the bacterium. For instance, DNA extracted from a penicillin-resistant mutant strain of pneumococcus will transform other pneumococci to heritable resistance, whereas DNA extracted from the parent sensitive strain will not. We infer that a bacterial mutation consists of a localized change in the genetically active DNA of the bacterium, which is arranged linearly in a linkage group or chromosome. In higher organisms the genotype may change in ways other than by mutation of a chromosomal gene; for instance, the number of chromosomes per nucleus may be doubled, or the organisms may lose some "self-replicating" cytoplasmic particle which cannot be synthesized *de novo*, e.g.,

a chloroplast. Some such changes may occur in bacteria also. Mutation of a chromosomal gene seems, however, to be the most common cause of change in bacterial genotype. Since in most cases the mechanism has not been investigated, I shall for the present purpose use "mutation" in a wide sense, without necessarily implying that mutation of a chromosomal gene is involved.

In higher organisms genetic recombination results almost always from a sexual process: in bacteria a variety of different methods have been discovered The one most closely resembling recombination through a sexual process was discovered in *Escherichia coli;* in this organism two bacteria conjugate, a part or the whole of the (solitary) chromosome of one partner passes into the other partner, to form a zygote, and crossing over between the two chromosomes follows, resulting in the production of a recombinant or hybrid bacterium, deriving some of its characters from one parent strain and some from the other. The segregation of particular characters, and by inference of the genes which control them, has enabled geneticists to chart the genes on a linear linkage map, which we believe is a representation of the bacterial chromosome. In this mating process in *E. coli* any bacterial character may recombine. There is also known among coliform bacteria a process in which conjugation between cells of two different strains results in one partner's acquiring just *one* character from the other; the characters thus transmissible are the ability to produce a colicine (an antibiotic active on certain coliform bacteria) and the "F" (fertility) character, required for ability to conjugate. We do not know whether the transfer of these characters (and of no others) during "conjugation" between certain strains results from the transfer of some hypothetical self-replicating cytoplasmic particle or from the transfer of a very short piece of chromosome bearing genes controlling these characters.

In addition to these processes of recombination by means of cell conjugation, we have available in the laboratory two methods of extracting genes from the disrupted cells of one strain (the gene donor strain) and transferring them into whole cells of a second, recipient, strain, where they recombine with the chromosomal genes of the recipient bacterium. In some genera (Pneumococcus, Haemophilus, etc.) one can dissolve the cells of the donor strain and extract the genes in the form of macromolecular deoxyribonucleic acid; this DNA will penetrate into cells of the recipient strain and recombine with the genes of these cells. As a result, a proportion of the treated cells acquire some new character from the donor strain from which the DNA was extracted and transmit this character to all their descendants. The process of genetic transduction by phage discovered in Salmonella is, we believe, essentially similar, but a phage particle is needed to carry the genetic material from one bacterium to the other; free DNA is ineffective. The cells of the donor strain are lysed, by growing phage in them; each bacterium bursts, liberating some hundreds of phage particles, some of which contain fragments of the chromosome of the lysed bacterium. When such a phage particle is absorbed by a bacterium of the recipient strain, the chromosome fragment from the previous host recombines by crossing over with the chromosome of the recipient bacterium, to produce a recombinant. In both these processes, transformation by DNA and phage-mediated transduction, only a small piece of foreign genetic material enters the cell, and contributes, by crossing over, to the genetic constitution of the recombinant, which is thus a hybrid deriving nearly all its characters from one parent, the recipient strain, and only one or a few from the other parent, the lysed, donor, strain.

Only a small minority of the phage particles in a Salmonella lysate carry bacterial genes from one bacterium to another in this way. However, in some phage strains each phage particle which attacks a sensitive bacterium has a good chance of establishing itself as a stable hereditary parasite in the bacterium, so that every one of this bac-

terium's descendants inherits the potentiality of liberating phage particles; such bacteria are called lysogenic. It is believed that in a lysogenic bacterium the phage genes are attached to the bacterial chromosome and are replicated *pari-passu* with it. When a bacterium becomes parasitized in this way, it may in consequence change its character in certain respects (*phage conversion*): for instance when non-toxin-producing strains of diphtheria bacillus acquire a heritable infection with certain phages they are converted into toxin-producing strains. The distinction between the transfer of bacterial genes to a new bacterium by a phage particle and the attachment of phage genes to the bacterial chromosome is not clear in some cases; we then cannot clearly distinguish between genetic transduction and lysogenic conversion: both, however, may be treated as instances of genetic recombination in bacteria.

The hereditary constitution of a bacterial strain may therefore change by mutation, or by any of several different processes of recombination. The comparison of bacterial strains of different hereditary constitutions, obtained by mutation or recombination, has been of great importance in the experimental study of bacterial infections of man and other animals.

GENETICS OF BACTERIAL VIRULENCE

I propose first to consider the role of the genetic constitution of the bacterium in determining its virulence when administered to an experimental animal of a particular species, and to defer consideration of the more complex case of naturally occurring infective disease. I use "virulence" here to mean the ability of the administered bacteria to multiply in the host and thereby to produce a pathological process; and, in a quantitative sense, to indicate the capacity of a bacterial strain to produce an infection from a small, rather than a large, challenge dose. The question which we seek to answer is what bacterial characters (in terms of bacterial physiology and anatomy) are required for virulence when the bacteria are administered to a particular host by a particular route.

The most clear-cut answers have been obtained in respect to bacterial characters found to be essential for virulence. Long before the days of the systematic study of bacterial genetics it was noted that, for instance, all virulent strains of pneumococcus and anthrax bacillus had the capacity to form capsules (at least in the environment provided by the host) and that all virulent Salmonella strains possessed the surface antigenic component conferring "smoothness." In many such instances the gain, or loss, by mutation, of the character associated with virulence can be easily demonstrated *in vitro;* for instance, in the pneumococcus one may, by the use of anti-"S" or anti-"R" sera obtain nonencapsulated variants from encapsulated strains and vice versa (see, for instance, [12]); the role of the serum is probably only to select spontaneously occurring mutants, not to induce them. The change in virulence which is found to accompany the change in bacterial character, e.g., encapsulation, is evidence that this character is essential for virulence, not merely an accidental concomitant of virulence in naturally occurring strains. The proof is completed when one can confer the character in question on a nonvirulent strain which lacks it, by some method of genetic recombination in which it is known that only a very small fraction of the genes of the recipient strain are altered. This was first accomplished by Griffith in his original work on "type-transformation" in pneumococci (11), but the genetic aspects of the phenomenon were not well understood until the transformation of a nonvirulent nonencapsulated pneumococcus to encapsulation, and virulence, was achieved *in vitro*, by the use of deoxyribonucleic acid extracted from an encapsulated strain.

An alternative approach to this problem of what characters are essential for virulence is to start with a virulent strain and to prepare from it a series of mutants, differing from it in respect to various biochemical or other characters, and to test the

virulence of these mutants. This was done in *Salmonella typhi* by Bacon, Burrows, and Yates (2), using the mouse as test host. (*Salmonella typhi* does not normally infect mice, and indeed a very large number of bacteria must be inoculated to produce a fatal infection in this animal, so that the system investigated was a highly "artificial" one; but it seems that the results obtained are also applicable to infections more nearly resembling those which occur in nature, e.g., infections of mice by *S. typhimurium*).

Mutants of the typhoid bacillus requiring an exogenous source of purine, para-aminobenzoic acid, or aspartic acid, were found to be of greatly diminished virulence (in that a much larger number of bacteria injected intraperitoneally was required to produce a fatal infection); whereas the virulence of mutants requiring various other growth factors differed little or not at all from that of the parent strain. It was shown that the peritoneal fluid of the mouse did not contain sufficient of the relevant growth factor (purines, PABA, or aspartic acid) to support the growth of the mutants and that the mortality of mice inoculated with, for instance, the PABA-requiring strain was increased if this substance was administered to the mouse. Back-mutants, no longer requiring any special growth factor, were isolated *in vitro* from some of these nutritionally exacting strains of diminished virulence; the reverted forms were found to have regained virulence, as well as the ability to dispense with the relevant growth-factor. These experiments establish that an essential criterion for virulence is that the nutritional requirements of the bacterium shall be satisfied by the pabulum provided by the relevant tissue of the host. This seems an obvious point, but one which seems to have been overlooked in the past. It seems to me that this line may, when applied in other situations, yield results of even greater interest. In the systems thus far studied the bacteria causing an infection multiply in the extra-cellular fluids of the host; it is relatively easy to obtain samples of such material and test whether it contains sufficient of a particular metabolite to support the growth of a nutritionally exacting bacterium. The case is otherwise when we are dealing with bacteria which multiply within the cells of the host. Testing the virulence of nutritionally exacting mutants of such bacteria may perhaps provide information as to the availability of various nutrients *within* the mammalian cell. Such information is essential in the rational design of antimetabolites for use in the chemotherapy of infections by bacteria which multiply intracellularly: if PABA were present in the intercellular fluids of the mouse, sulfonamide therapy would be ineffective in this animal.

We can thus define certain characters, in particular the presence of some components at the bacterial cell surface and the capacity to grow without an external source of certain metabolites, which are necessary for virulence in particular systems. Such characters are not, however, sufficient for virulence, as is evident from the fact that many bacterial species are virulent for one mammalian host but not for another; for instance, most strains of *Salmonella typhimurium* after serial passage became highly virulent for mice, the LD_{50} by intraperitoneal injection being commonly less than ten bacteria, whereas the LD_{50} for the closely related organism *S. paratyphi B*, even after serial passage, is in the region of 10^7 bacteria. These two bacteria have identical somatic antigens and are both nutritionally nonexacting, and the reason for the difference in their mouse virulence is unknown, the characters relied upon for their differentiation, e.g. their flagellar antigens, being almost certainly not concerned in virulence. It is possible that the bacterial geneticist can help in the search for the relevant difference between the two species, for one can transfer characters, one at a time, from *S. typhimurium* to *S. paratyphi B*, or vice versa, by genetic transduction, using bacteriophage as a vector of genetic material. Since we can thus, in a sense, hybridize the species, it is at least theoretically possible that we might

discover the bacterial character concerned in the difference in mouse pathogenicity by examining the pathogenicity of hybrid strains showing various combinations of parental characters.

The problem of host-specificity, which is in a sense the problem of the less obvious bacterial characters required for virulence, is accessible to experiment in such cases, when a susceptible experimental animal is available. A successful analysis might then assist us to discover the basis of the ability of, for instance, the gonococcus to infect man, but none of the usual laboratory animals.

BACTERIAL GENETICS AND ACTIVE IMMUNIZATION

In many bacterial infections the disease process is terminated, and the host is rendered partly or totally immune to further attack, by a specific immunological response of the host to one or more antigens of the bacterium, that is, by the production of specific antibodies; and we may wish to protect susceptible persons by active immunization. In a few cases, in particular diphtheria and tetanus, we can immunize with a well identified bacterial product, but in most cases no such single "protective antigen" is available, and we must rely on the administration of whole bacterial cells (or cultures), either killed, or living but avirulent. The importance of a genetic approach to the selection of strains of good immunizing potency for use as dead vaccine is evident, and I shall not discuss it here. In some infections, however, only the administration of live vaccines has been found effective in immunization. The problem then is to find a bacterial strain which can be relied upon to give an adequate immunological stimulus to the host, but never itself to cause a serious pathological process. We require a nonvirulent strain which can be guaranteed *never* to revert to virulence; if even a single virulent bacterium arising by reversion were present in the vaccine inoculum it would presumably multiply in the inoculated host and cause a progressive infection. Bacterial strains which can be used as live vaccines have been obtained, in the main, empirically. The BCG strain of tubercle bacillus and certain strains of the plague bacillus have been extensively used for human immunization, despite lack of knowledge of what bacterial characters are responsible for their lack of virulence. It is interesting to contrast this with the position in respect to immunization of animals against anthrax. It is known that all virulent strains of *Bacillus anthracis* produce capsules *in vivo*, and Sterne (18) showed that all such strains also produced capsules *in vitro*, provided they were grown in the presence of serum and of a raised concentration of carbon dioxide. The capsule of the anthrax bacillus, which is composed of a polymer of D-glutamic acid, protects the bacillus against phagocytosis, and this presumably is why its presence is essential for virulence. Sterne (18) showed that it was easy to isolate nonencapsulated variants, or mutants, from virulent strains, and that live spore suspensions of such variants were satisfactory immunizing agents. Live vaccines of this type have been very widely used for a number of years; the vaccine strain has never been known to revert to virulence. We have here an example of the successful use of an avirulent mutant as a live vaccine, in which we know the physiological basis for the absence of virulence. We do not, however, know why these nonencapsulated mutants of *B. anthracis* never regain their encapsulation. In other genera, e.g., in the pneumococcus, some nonencapsulated variants have a low, but detectable, rate of back mutation to encapsulation, so that injection of very large numbers of the nonencapsulated strain into mice sometimes causes death, an encapsulated culture being recovered at post mortem examination. Very little systematic genetical work has been done on the *Bacillus* group, and no recombination method is available for testing whether, for instance, the stable nonencapsulated mutants result from loss of a whole segment of chromosome, as appears to be the case in some nonreverting biochemical mutants in Salmonella.

The successful use as a live anthrax vaccine of a nonvirulent strain which owes its lack of virulence to a mutation which does not impair its immunizing efficacy suggests that it might be possible to prepare equally safe live vaccines in other genera. In Salmonella, for instance, there are available the types of biochemical mutant mentioned above, which are nonvirulent because of their nutritional requirements and yet retain the normal antigenic structure. In *S. typhimurium* the use of some such mutants as vaccines has been shown to confer some protection on mice. It might be possible to prepare similar mutants in, for instance, the tubercle bacillus. However, the great difficulty in getting properly controlled tests on the efficacy in man of even existing vaccines, e.g., typhoid vaccine and BCG, suggests that even if such 'made to measure' strains were available it would be difficult to determine their value for human immunization.

BACTERIAL GENETICS AND CHEMOTHERAPY OF BACTERIAL INFECTIONS

It frequently happens that bacterial infections cannot be successfully treated with certain antibiotics or other chemotherapeutic agents, because the infecting bacteria are, or during treatment become, resistant to the agent, even though they belong to a species regarded as universally sensitive to the agent concerned when it was first introduced. This replacement of populations of sensitive bacteria by populations of resistant ones provides a beautiful, but inconvenient, illustration of the phenomenon of evolution through natural selection, and because of it the genetic analysis of drug-resistance in bacteria is of great practical importance. It has also been of theoretical importance in the development of bacterial genetics, because of the need to resolve the conflict as to the mechanism by which a bacterial strain "becomes resistant" when cultivated in the presence of an antibacterial agent. In earlier years it was generally believed that the heritable increase in resistance found in a bacterial culture which has been "trained" by growth in the presence of an anti-bacterial drug resulted from a specific change (resistance) in the hereditary constitution of the individual bacteria directly caused by the action of the drug. The researches of the bacterial geneticists, culminating in the elegant method of indirect selection (Lederberg & Lederberg [14]) have, however, proved that heritable resistance of a culture to a drug can arise from the selection by the drug of resistant mutants, resulting from spontaneous mutations which occur in the presence or the absence of the drug. In my opinion spontaneous mutation and selection can account for all the observed phenomena of acquired heritable drug-resistance in bacteria; there is, I believe, no instance in which it has been conclusively proved that heritable resistance has been produced by a specific change in the hereditary character specifically induced by the drug concerned.

One may ask what bearing this theoretical conclusion has on the practical problems of drug-resistance. The nature of the problem varies from one system to another. Only in a few situations does it happen that an infecting population of sensitive bacteria are replaced during treatment by resistant bacteria which are descendants of the original sensitive ones, rather than the result of a secondary infection. However, this may occur in several different kinds of bacteria, when streptomycin is used. There is every reason to suppose that the mechanism then is simply the selection of streptomycin-resistant mutants, resulting from spontaneous mutations occurring during the multiplication of the sensitive bacteria in the patient. The clearest evidence is from the experiments of Garrod (8) in the early days of streptomycin therapy. He examined the urine of patients suffering from acute urinary infections caused by streptomycin-sensitive coliform organisms. Very large numbers of bacteria, e.g., 10^{10}, obtained by concentrating the bacteria from urine collected before treatment, were plated in streptomycin agar; a small number of colonies developed in some cases, indicating that streptomycin-resistant organisms, pre-

sumably mutants, were present in very small numbers even before treatment was begun. The probability of cure by streptomycin seemed (in the limited number of cases) to be inversely related to the frequency of these resistant cells. There are other antibiotics in which treatment may fail because of the appearance of resistant mutants during treatment, for instance erythromycin. However, in the case of penicillin and various other agents there is, in most infections, no such risk, or only a negligible risk. The problem of drug resistance, for instance of penicillin resistance in staphylococci, is then that of patients who are primarily infected, or superinfected during treatment, by resistant strains which have become common in hospitals and elsewhere since the agents concerned (penicillin, etc.) came into general use. In the case of yet other pairs of drug and bacterium, there is, surprisingly, no problem of drug resistance; for instance, no human infections by penicillin-resistant strains of group A streptococci or *Treponema pallidum* have been reported, as far as I know.

The study of the genetics of drug resistance explains some of these varying situations. The emergence of streptomycin-resistant descendants in the course of streptomycin therapy can be explained by the laboratory observation that bacteria of many different genera undergo mutation to high resistance to streptomycin, in a single step, at rates of about 10^{-10} per bacterium per generation, these mutants commonly resembling their parent in most other characters including virulence. In the case of penicillin, on the other hand, laboratory studies show that only a small increase in resistance can be achieved by a single mutation and that mutations which confer increased resistance often also cause decreased virulence. We can thus explain the difference between the streptomycin and the penicillin situations by showing that a particular kind of mutation occurs at a measurable rate in the one case and not in the other. It remains to be discovered why streptomycin-sensitivity can be lost by a single mutative step, but not penicillin-sensitivity; this is a problem in physiological genetics which is not likely to be solved until we know a lot more about the mode of action of these antibiotics.

We are less well informed as to why, for instance, penicillin-resistant coagulase-positive staphylococci have become prevalent, whereas penicillin-resistant group A streptococci have not—despite the fact that mouse-virulent penicillin-resistant variants have been obtained from group A streptococci in the laboratory (Rosendal [16]), whereas penicillin-resistant staphylococci of the kind which cause trouble in practice (i.e., strong penicillinase-producers) have never been conclusively shown to arise from sensitive strains, though the reverse change, mutative loss of ability to produce penicillinase, and so of penicillin resistance, occurs frequently (Barber [3]). A possible explanation is that a small minority of penicillin-resistant coagulase-positive strains of staphylococcus existed before penicillin came into use and have since become prevalent as sensitive strains are eliminated; but that no such strains of group A streptococci existed and that to obtain penicillin resistance in this organism, without loss of virulence, requires a large number of successive mutations, first-step mutants not being sufficiently penicillin-resistant (or virulent) to be selected during the penicillin treatment of infections or in penicillin-contaminated environments.

An understanding of the genetic mechanisms of drug-resistance can be of practical value in this field. When the use of an agent may fail because of the appearance of resistant mutants during treatment, e.g., in streptomycin therapy, the simultaneous use of a second agent, resistance to which can only be acquired by a separate mutation, is theoretically valid and seems to have been successful in practice. If it can be shown that the replacement of sensitive organisms by resistant ones during treatment results, in a particular situation, not from selection of resistant mutants but from secondary infections by resistant strains, then the clinician

knows that precautions against cross-infection must be improved. The increasing prevalence of antibiotic-resistant strains in hospitals and the community at large is a problem in population genetics which requires much further study. On theoretical grounds it seems probable that if the use of, e.g., penicillin, was abandoned, not only in medicine but also in animal husbandry, etc., penicillin-sensitive strains of staphylococcus would gradually become more prevalent again, that is there would be a trend back toward the population equilibrium of sensitive and resistant strains which presumably existed before the introduction of the agent. However, it is very unlikely that such a step could be taken, even if it were considered desirable; it would seem advisable to discover more about the way in which resistant strains are selected. Gould (10) has recently shown that the air in casualty departments and the noses of hospital staff are contaminated with significant amounts of penicillin, and it seems possible that such contamination, which is in theory avoidable, and the selective conditions for resistant staphylococci which it creates in the anterior noses of hospital staff, is a much more important cause of the prevalence of penicillin-resistant strains than is the selective medium provided by patients under treatment.

USE OF GENETICALLY "LABELED" BACTERIAL STRAINS IN THE INVESTIGATION OF INFECTIONS

Minor bacterial characters, genetically stable and in themselves without effect on pathogenicity, may serve to split up a bacterial species into a series of sub-units, as for instance the phage-types of *Salmonella typhi*. This is often of great practical value. Minor antigenic characters, pattern of phage sensitivity ("phage-type"), and capacity to ferment particular sugars have been the most commonly used characters. It now seems that a number of other characters which have been of interest to the bacterial geneticist may be of value to the epidemiologist. For instance, strains of *Escherichia coli* may be differentiated by their sensitivity (inhibition of growth) to various amino acids (Rowley [17]), and strains of enteropathogenic *E. coli* and of *Shigella sonnei* by their production of different colicines (i.e., antibiotics active on certain strains of coliform bacteria); the latter character appears to be of value in epidemiological analysis, (Frédéricq, Betz-Bareau, and Nicolle, [7], Abbott and Shannon [1]). The gonococcus and the tubercle bacillus have thus far received little attention from bacterial geneticists, presumably because they are technically inconvenient material; but a search for suitable characters might make it possible to assign strains of gonococcus and tubercle bacillus (human variety) to types, which might be of use in attempts to control the diseases concerned.

One may also use these banal characters for the preparation, in the laboratory, of genetically labeled sub-strains of a particular strain, for use in experimental investigations of infection; recognizable sub-strains may be obtained by selection of mutants or by some method of genetic recombination. If several such sub-lines of a strain do not differ in pathogenicity or in rate of multiplication *in vivo* (if, in fact, the genetic "label" is no more than a label), they can be used to test, in a particular system, certain general hypotheses as to the mechanism of the production of infection. In many systems a very large number of live bacteria must be administered to elicit an infective response (fatal infection, local lesion, etc.) in 50 per cent of the hosts tested (i.e., the ED_{50} is large); this may be true even when the bacterial strain has been repeatedly passaged to select a variant of the maximum possible virulence. For instance, when mice are given inoculations intraperitoneally of *Salmonella paratyphi B*, the ED_{50} for a fatal infection is about 7,000,000 bacteria. There are two general hypotheses about this sort of situation, which I shall state as applied to the example selected. According to one, there is for each mouse a *Minimum Lethal Dose* (M.L.D.), i.e., a number of injected

bacteria which is just sufficient to swamp its defense mechanisms, so that if any larger number is inoculated all or many of them will multiply and cause a fatal infection; in the example stated the modal value of this M.L.D. is 7,000,000. The alternative hypothesis, of *Independent Action* (I.A.), states that every inoculated bacterium has a small chance of multiplying in the mouse so as to cause a fatal infection, and that for each individual bacterium inoculated this probability is unaffected by the number of other bacteria inoculated: if each inoculated bacterium has only a one in ten million chance of multiplying, it will be necessary to inoculate about seven million bacteria to achieve a one in two probability that one, two, or more of them will multiply and kill the mouse. Either of these hypotheses can account for the observed relation between dose and mortality. They differ in the way in which they account for fatal infections resulting from inocula just sufficient to kill, say one LD_{50} or less: according to the hypothesis of the M.L.D., mice dying as a result of inoculation of one LD_{50}, i.e., 7,000,000 bacteria, die because many of the inoculated bacteria multiply; but, according to the hypothesis of I.A., they die because a very small number, most probably one, of the inoculated millions chance to multiply. This antithesis permits an experimental test, by the use of an inoculum containing equal parts of several labeled sub-strains, say *a*, *b*, and *c*. If the hypothesis of the M.L.D. is applicable, mice which die from inoculation of one LD_{50} (comprising 2,700,000 each of *a*, *b*, and *c*) die as a result of multiplication of *many* of the inoculated bacteria, and the infecting population will therefore comprise all three sub-strains in about equal numbers; but, if the hypothesis of I.A. applies, most mice dying after inoculation of one LD_{50} (7,000,000 bacteria) die as a result of the multiplication of only one bacterium, and the infecting population will therefore consist of only *one* sub-strain, so that some mice will give, at post mortem, a pure culture of sub-strain *a*, others of sub-strain *b*, and so on. This mixed-inoculum experiment was, I believe, first used by Zelle, Lincoln, and Young (21), who infected guinea pigs with anthrax by inhalation of spores. The spore suspension contained equal parts of two variants differing in colonial morphology, but of equal virulence; many guinea pigs which died yielded at post mortem pure cultures of one variant or the other, as predicted by the hypothesis of independent action. Meynell and Stocker (15) applied the test to mice inoculated intraperitoneally with *Salmonella paratyphi B;* they used variants differing in their flagellar antigens, obtained by transduction. The results obtained were not clear-cut, but were interpreted as indicating the applicability of the hypothesis of independent action, with some modifications. Gorrill (9) has used this kind of test in an investigation of the effects of intravenous inoculation of staphylococci into mice; the mice survive the inoculation of large numbers of bacteria but develop renal abscesses, the total number of abscesses being very much fewer (about 100,000-fold) than the number of bacteria inoculated. When the inoculum contained a mixture of streptomycin-sensitive and streptomycin-resistant sub-strains, the large majority of the resulting abscesses gave pure cultures, of either sensitive or resistant bacteria, each mouse having abscesses of both types. This presumably indicates that the large majority of inoculated staphylococci are disposed of by the mouse but that a small minority produce abscesses in the kidney, each such abscess resulting from the multiplication of a single bacterium.

The use of genetically labeled strains may perhaps prove useful in the investigation of many other bacterial infections.

BACTERIAL GENETICS AND EPIDEMIOLOGY

The epidemiologist is concerned with the question of what natural mechanisms regulate the degree of virulence for the normal host of strains of pathogenic species and of the role, if any, of changes of virulence in the production of epidemics, etc. For most bacterial infections of man we have but little

information on this matter (see discussion by Wilson and Miles [20]) and cannot easily obtain more, through lack of a reliable experimental measure of "virulence for man." One can, however, make a theoretical analysis and correlate it with some relevant observations; such an analysis must be based on the results of experiments on the genetics of virulence measured in an experimental animal, even though these have of necessity involved highly "artificial" conditions, e.g., inoculation by intraperitoneal injection. Such studies have established that the virulence of a strain, as measured by a particular test, is subject to quantitative and qualitative variation; that serial passage of a strain from host to host commonly results in some increase in virulence for the host species concerned, it may be presumed because the procedure selects mutants of increased virulence; that strains of enhanced virulence thus obtained may be of unchanged, or even diminished, virulence when tested on a different host species, or by a different route. It is also known that strains maintained by serial culture *in vitro* often, but not always, decrease in virulence, the diminished virulence frequently reflecting a genetic heterogeneity, with an increasing proportion of the culture consisting of nonvirulent, or less virulent, variants. The population genetics of these changes have not been fully investigated; spontaneous mutation suffices to explain the origin of the less virulent forms, but it is probable that their rapid increase within a strain results from their better adaptation to the *in vitro* environment, for instance, from more rapid growth than the parental strain. When a strain is maintained in a liquid medium the genetically different components, e.g., smooth and rough, may interact in complex ways, the metabolic products of one component affecting the growth or survival of another, as shown by the extensive researches of Braun and his collaborators in *Brucella* (see Braun [4]). In a few instances, continuous *in vitro* passage of a strain of low virulence results in an increase in virulence (Felton and Dougherty [6]).

When considering the mechanisms regulating the virulence of strains in nature, rather than in the laboratory, one must first consider the ecological situation. Some human infections, e.g., gas gangrene, are purly "accidental" from the point of view of the bacterial strain, since the infection does not contribute to the dispersal of the strain; in such systems virulence for man is an accidental concomitant of some feature, e.g., the production of an enzyme, which may have survival value in the normal ecological niche, e.g., the soil. I shall discuss the opposite case, i.e., bacteria which multiply only as parasites of man, e.g., the typhoid bacillus. It seems clear that in the long run natural selection will here favor a parasite which seldom or never kills its host, and that there will be an evolutionary trend toward "low virulence" in this sense. Acute cases of typhoid fever mostly result from the dissemination of *Salmonella typhi* by so-called carriers, who in fact suffer from a chronic infection, commonly of the gall bladder, which causes only mild symptoms; it appears, then, that the typhoid bacillus has evolved to a state in which it is well adapted to prolonged coexistence with a human host, and it is perhaps surprising that when it infects a new host it should cause such a severe, frequently fatal, disease as typhoid fever. I suggest that the explanation may be that the typhoid bacillus, though well adapted to cause a chronic, nonlethal infection of, for instance, a previously damaged gall bladder, is only enabled to reach this site by its capacity to cause a generalized infection when it infects a fresh host; natural selection will then insure that the species retains sufficient virulence to do this, and the death of a proportion of newly invaded hosts may be, for the species, an unfortunate but unavoidable consequence of this necessary degree of virulence.

Some typhoid "carriers" excrete mainly rough typhoid bacilli, which are avirulent and therefore unable to infect a new host. This illustrates the point that mutations to nonvirulence occur in nature, as well as in

the laboratory. ***Bacillus anthracis*** is probably in nature an obligate pathogen of animal hosts; yet it too produces completely nonvirulent mutants in nature, as well as in the laboratory, for Chu (5) was able to isolate nonencapsulated, and therefore avirulent, strains of *B. anthracis* from consignments of goat skin which also yielded virulent strains, and also from soil experimentally contaminated with blood from animals dying from anthrax.

In addition to mutants of diminished virulence, which will be eliminated in due course because of their inability to infect new hosts, other classes of mutants will arise in infecting strains. When a bacterial strain is kept in continuous growth in the laboratory, the proportion of mutants which grow neither faster nor slower than the parent strain gradually rises; this presumably happens also in strains which are multiplying *in vivo*. However, there are theoretical reasons (see above, hypothesis of independent action) for supposing that in nature the infection of a new host usually results from the multiplication in it of only one, or a small number, of the bacteria shed by the previous host (in the case of typhoid fever there is indeed epidemiological evidence—Kehr and Butterfield [13]—that many infections result from the ingestion of only a single typhoid bacterium); in consequence, each passage from host to host will generally entail the elimination of all the mutants which had accumulated in the strain in its previous host, in the same way that they are eliminated when a strain is "purified" in the laboratory by picking a single colony. There are observations which suggest that this occurs even in the case of highly mutable characters such as H antigen phase change in *Salmonella typhimurium* (see discussion in Stocker [19]).

The specific antibody produced by the host contributes to the elimination of the bacteria in "self-limited" infections; mutants lacking the original surface antigen, but of undiminished virulence, would be unaffected by this antibody, and so presumably would be at a relative advantage. It appears that the relapses in experimental relapsing fever result from the capacity of the Treponema strains concerned to produce a series of serologically distinct variants which are unaffected by protective antibodies directed against the original strain. It is perhaps surprising that the same phenomenon is not encountered in other genera, for instance the Salmonella.

One may ask what part, if any, the various mechanisms of genetic recombination in bacteria play in nature, and in particular in the evolution and epidemiological behavior of bacterial pathogens. There is good evidence that pathogenic strains may in nature become, or cease to be, lysogenic for particular phages, and since in some cases changes in virulence result, this process evidently has some importance. However, there is, in my opinion, at present no evidence that the other known mechanisms (mating, phage-mediated transduction, transformation by DNA, transfer of F factor and of colicinogenicity) have a role of any importance in epidemiology, since spontaneous mutation and selection can account for the observed instances of changes of hereditary character; their role in the evolution of bacteria is equally problematical. Their chief interest in the study of infectious disease is as tools for the investigation both of infectious disease and of the underlying problems of bacterial variation.

REFERENCES

1. ABBOTT, J. D., and SHANNON, R. A Method for Typing *Shigella sonnei*, Using Colicine Production as a Marker. J. Clin. Path., 11:71–77, 1958.
2. BACON, G. A.; BURROWS, T. W.; and YATES, M. The Effects of Biochemical Mutation on the Virulence of *Bacterium typhosum:* the Loss of Virulence of Certain Mutants. Brit. J. Exper. Path., 32:85–96, 1951.
3. BARBER, M. Drug Resistance of Staphylococci with Special Reference to Penicillinase Production. *In:* Drug Resistance in Micro-organisms: Mechanisms of Development, pp. 262–79. London: Churchill, 1957.

4. BRAUN, W. Bacterial Genetics. Philadelphia: Saunders, 1953.
5. CHU, H. P. Variation of *Bacillus anthracis* with Special Reference to the Non-capsulated Avirulent Variant. J. Hyg., Camb., **50**:433–44, 1952.
6. FELTON, L. D., and DOUGHERTY, K. M. Studies on Virulence II. The Increase in Virulence *in Vitro* of a Strain of Pneumococcus. J. Exper. Med., **39**:137–54, 1924.
7. FRÉDÉRICQ, P.; BETZ-BAREAU, M.; and NICOLLE, P. Typage des souches d'*Escherichia coli* de gastro-entérite infantile par recherches de leurs propriétés colicinogènes. C. R. Soc. Biol., **150**:2039–41, 1956.
8. GARROD, L. P. The Reactions of Bacteria to Chemotherapeutic Agents. Brit. M. J., **1**:205–10, 1951.
9. GORRILL, R. H. The Establishment of Staphylococcal Abscesses in the Mouse Kidney. Brit. J. Exper. Path., **39**:203–12, 1958.
10. GOULD, J. Environmental Penicillin and Penicillin-resistant *Staphylococcus aureus*. Lancet, **1**:489–93, 1958.
11. GRIFFITH, F. The Significance of Pneumococcal Types. J. Hyg., Camb., **27**:113–59, 1928.
12. ———. Serological Races of Pneumococci. *In:* A System of Bacteriology in Relation to Medicine, pp. 201–25. London: Medical Research Council, 1929.
13. KEHR, R. W., and BUTTERFIELD, C. T. Notes on the Relation between Coliforms and Enteric Pathogens. Pub. Health Rep., **58**:589–607, 1943.
14. LEDERBERG, J., and LEDERBERG, E. M. Replica Plating and Indirect Selection of Bacterial Mutants. J. Bact., **63**:399–406, 1952.
15. MEYNELL, G. G., and STOCKER, B. A. D. Some Hypotheses on the Aetiology of Fatal Infections in Partially Resistant Hosts and Their Application to Mice Challenged with *Salmonella paratyphi B* or *Salmonella typhimurium* by Intraperitoneal Injection. J. Gen. Microbiol., **16**:38–58, 1957.
16. ROSENDAL, K. Investigation of Penicillin-resistant Streptococci Belonging to Group A. Acta Path. microbiol. Scandinav., **42**: 181–88, 1958.
17. ROWLEY, D. Interrelationships between Amino-acids in the Growth of Coliform Organisms. J. Gen. Microbiol., **9**:37–43, 1953.
18. STERNE, M. Variation in *Bacillus anthracis*. Onderspoort J. Vet. Sc., **8**:274–349, 1937.
19. STOCKER, B. A. D. Measurement of Rate of Mutation of Flagellar Antigenic Phase in *Salmonella typhimurium*. J. Hyg., Camb., **47**:398–413, 1949.
20. WILSON, G. S., and MILES, A. A. Herd Infection and Herd Immunity. *In:* Topley and Wilson's Principles of Bacteriology and Immunity, 4th ed., pp. 1437–60. London: Edward Arnold, 1955.
21. ZELLE, M. R.; LINCOLN, R. E.; and YOUNG, G. A. Respiratory Pathogenicity of *Bacillus anthracis* Spores. II. Genetic Variation in Respiratory Pathogenicity and Invasiveness of Colonial Variants of *B. anthracis*. J. Infect. Dis., **79**:247–53, 1946.

Genetic Aspects of Tissue Transplantation and Tolerance

RAY D. OWEN*

Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tenn.

Nowhere is the diversity among the individuals of a species more evident than in the field of tissue transplantation. Surgeons today consider it axiomatic that a skin graft from one person will be rejected by any other person, unless the donor and recipient are identical twins. In fact, acceptance of skin grafts exchanged between two persons now has medicolegal status as evidence that the two persons in question are identical twins. The fact that identical twins constitute the universally recognized exception to the incompatibility of tissue transplants between individuals reflects the recognition that this kind of variation is inherited; it is their genetic identity that provides these twins with a unique basis for tissue compatibility (cf. Medawar [51]).

The mechanism of tissue rejection is generally accepted as immunologic. An individual who rejects a tissue transplant is so sensitized by this experience that he rejects a second transplant from the same donor more rapidly than he did the first. This "immunity" can be passively transferred from one experimental animal to another by injecting spleen or lymph node cells from the immune animal into a normal recipient of the same inbred line (52). With the establishment of these immune cells, the injected animal acquires the ability to give the immune, "second-set" response to transplants of the sort against which the cells were sensitized.

We have, therefore, a common appreciation of individuality in tissue transplantation, individuality based on such great diversity of genetic constitution that two individuals, unless they are identical twins or members of the same inbred line, are rarely alike. The genetic variation is expressed as antigenic differences among individuals. Donor tissue possessing one or more antigens different from those of its host calls forth an immune response in the host and sets in train processes leading to the destruction of the grafted tissue. The immunologic part of this system is a matter of lively interest, but our attention here must be directed toward the nature and genetic control of materials responsible for initiating the immune response, and not with the response itself.

The speakers at this symposium were urged to generalize in the treatment of their subjects. I am in sympathy with that estimate of what is proper here, but hope that the many scholars who have contributed in important ways to this field, and who are currently making contributions, will understand and forgive the uneven recognition that is inherent in a paper of this kind. The literature citations include several reviews and research reports that do a better job of giving recognition where it is due.

GENERAL CONSIDERATIONS

As one might expect, recognition of individual diversity in tissue transplantation

* On leave, 1957–58, from the Division of Biology, California Institute of Technology, Pasadena, Calif.

† Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

antedates any attempt to analyze or explain this diversity in modern genetic terms. Among the earlier clear encounters with the phenomenon were experiences with incompatibility in parabiosis, in the mid-19th century (see Finerty [21]). As early as 1905 Ehrlich recognized immunization by a tumor transplant in experimental animals, and in 1912 Schoene (64) reported that rabbits given injections of tissue preparations from other rabbits destroyed subsequent skin grafts from these donors more rapidly, whereas skin "autotransplanted" from one site to another on the same animal was not affected. Tyzzer, in 1909 (76) concluded that susceptibility to a particular carcinoma was inherited, but this inheritance did not seem to obey Mendelian laws. Almost all hybrids between a resistant and a susceptible group of mice were susceptible to the test carcinoma, as though susceptibility were dominant in a Mendelian sense. But in the next two generations, no susceptible mice were found. This disappearance of apparent dominance was difficult to comprehend in Mendelian terms.

In 1914, Dr. C. C. Little (38), then just completing his graduate study at the Bussey Institution, published a note in *Science* showing that the kinds of ratios encountered by Tyzzer and by Little in Tyzzer's laboratory were to be expected if susceptibility and resistance resulted from the interaction of numerous different dominant factors. In 1916, Little and Tyzzer (44) reported on a series of 629 mice given inoculations of a particular tumor and developed a concept for the genetic basis of tissue incompatibility in modern terms. Dr. Little has reviewed the subject to 1941 (40), and Snell (69) has given a brief account of the development of the field of tumor transplantation at the Jackson Memorial Laboratory, from which many of the most important contributions to this field have come.

The transplantation of normal tissues was subjected to the same kind of analysis by Little and Johnson in 1922 (42) and extended by other workers, particularly by Loeb and Wright (45). Rogers (63) presents a brief review of parallel and concurrent developments, particularly in Germany. Thirty years ago, therefore, at least the broader outline of current genetic concepts of tissue incompatibility had been defined.

RESISTANCE TO TUMOR TRANSPLANTS

The relative ease of tumor transplantation and the availability of a conspicuous and objective assay for the success or failure of the transplant have made tumor transplantation a most productive technique for the evaluation of the genetic control of tissue incompatibility. In general, a host can cause a strongly incompatible tumor to regress, whereas a relatively compatible tumor grows progressively and causes the death of its host. The availability of a large selection of reasonably stable mouse tumors and the increasing availability of highly inbred lines of mice, with virtually identical genetic constitutions within each line and marked genetic differences between lines, have provided other necessary components of a productive test system. On the other hand, the occurrence of escapes of either host or tumor, with different frequencies depending mainly on the particular host-tumor combinations under study, often complicates this kind of analysis.

Table 1 illustrates the classic experience in this field. Little and Tyzzer found that a mammary adenocarcinoma of a strain of Japanese waltzing mice grew progressively when transplanted to other mice of the same strain. Common mice were all resistant to implants of this tumor. Hybrids between susceptible and resistant mice were virtually all susceptible, suggesting a "dominance of susceptibility" to the tumor. Of the F_2 hybrids, however, only three were susceptible out of a test population of 183. All backcrosses to the susceptible parent were susceptible, whereas backcrosses to the resistant parent were all resistant.

The genetic basis for this kind of result is conceived as follows: Suppose that the tumor has a gene, A, controlling the presence of an antigenic specificity that will induce an immune response leading to regression of the

tumor in any host lacking this gene (*aa*). If the Japanese waltzing strain is *AA* and common mice are *aa*, their hybrids will be *Aa*, and the tumor will grow in them. The F_2 hybrids would segregate as three "susceptible" (having the *A* gene either homozygous or heterozygous) and one resistant (*aa*). Obviously, the assumption that a single gene difference between the parents is responsible for this tumor-host relationship does not explain the low frequency of susceptible mice in F_2. Suppose, however, that *two* independent dominants are present in the tumor, the absence of either of which in the host would make him resistant. The hybrids, *AaBb*, would again be susceptible, but in F_2 only nine of sixteen would be expected to have both *A* and *B* and therefore to be susceptible to this tumor. The "percentage susceptible" in F_2 would have dropped 56.25. Again, the assumption of two factors is inadequate to explain the experimental data, but a start has been made in the right direction.

TABLE 1

GROWTH OF TUMOR JWA IN JAPANESE WALTZING MICE, IN COMMON MICE, AND IN VARIOUS HYBRIDS BETWEEN THESE TWO*

Stock	+	−	Per Cent +
Japanese waltzing mice	38	0	100.0
Common mice	0	99	0.0
F_1 hybrids	61	1	98.4
F_2 hybrids	3	180	1.6
F_1 hybrids × Japanese waltzing mice	63	0	100.0
F_1 hybrids × common	0	78	0.0

* From Little, 1941. *In:* G. D. SNELL (ed.), "Biology of the Laboratory Mouse," p. 280.

TABLE 2

THE RELATION BETWEEN THE PERCENTAGE OF MICE SUSCEPTIBLE TO A TRANSPLANTED TUMOR AND THE NUMBER OF GENES RESPONSIBLE FOR THE SUSCEPTIBILITY

Pairs of genes the simultaneous presence of which is needed	Per cent susceptible in F_1	Per cent susceptible in F_2	Per cent susceptible in backcross of F_1 × susceptible parent	Per cent susceptible in backcross of F_1 × nonsusceptible parent
1	100	75.0	100	50.0
2	100	56.2	100	25.0
3	100	42.2	100	12.5
4	100	31.6	100	6.2
5	100	23.7	100	3.1
6	100	17.8	100	1.6
7	100	13.3	100	0.8
8	100	10.0	100	0.4
9	100	7.5	100	0.2
10	100	5.6	100	0.1
11	100	4.2	100	0.05
12	100	3.1	100	0.02
13	100	2.3	100	0.01
14	100	1.7	100	0.005
15	100	1.3	100	0.002
16	100	1.0	100	0.001

* From Little, 1941. *In:* G. D. SNELL (ed.), "Biology of the Laboratory Mouse," p. 283.

Table 2 shows that the expected percentage susceptible in F_2 falls in a series $(3/4)^n$ as n, the number of independent pairs of genes postulated for this kind of

system, increases. The observation of 1.6 per cent susceptible in the F_2 of the classic case would be consistent with the assumption of about fourteen gene loci assorting independently and acting as assumed. Furthermore, in the backcross, the probability of susceptible individuals is lower for each number of postulated gene pairs, and falls in a series $(1/2)^n$. All backcrosses to the susceptible parent would be susceptible, but only five animals in 1000 in the backcross to the resistant parent would be expected to be susceptible, if fourteen gene pairs are segregating. "Resistant backcross" ratios have on the whole given results compatible with the conclusions from F_2 analysis. Both linkage and the presence of "weak" histocompatibility alleles conferring incomplete protection on the host toward a tumor result in underestimation of the true number of genes concerned.

The mice available for the early studies of compatibility in tumor transplantation were not good inbred lines, in the modern sense, and the groups of mice providing the basis for the segregating populations were very different from one another. In general, later estimates of the number of gene loci concerned with the transplantation of particular tumors have been lower than the fourteen or so suggested in the classic study just cited. Soon afterward (39) data on a sarcoma of the Japanese waltzing mice indicated that only four or five genes were needed. Later, Little and Strong (43) studied two transplanted adenocarcinomas of DBA mice, in hybrid generations after crosses with A mice, and concluded that one of these tumors required two and the other three genes. Simultaneous reactions of animals to the two tumors showed that the two genes important to the first tumor were also involved in the second; only one additional locus was required for the second. In 1926, Strong reported a "one-gene" ratio in another combination and later a "four-gene" ratio in which one of the genes appeared to be sex-linked. An approach had been developed, therefore, through which not only could the number of genes involved in tumor transplantation compatibility be estimated, but the contributions of particular genes could be identified.

During the late 1940's, Dr. George Snell and his colleagues developed efficient methods for the identification and manipulation of particular loci involved in histocompatibility. At least two components of this important methodological contribution should be singled out for mention here. One is the development of "isogenic resistant" lines that may be assumed to differ only at the locus under study; the second is the use of linked, visible markers to follow the segregation of the locus in question. The method applied to the development of isogenic-resistant (IR) lines of mice is as follows: Given an inbred strain of mice designated as strain A and a tumor originating in this strain, to which the strain is susceptible, mice of strain A are crossed to any mice found to be resistant to the tumor. An F_2 generation is produced, and the tumor is inoculated into mice of this generation. Susceptible mice will be killed by the growing tumor, and only resistant mice will survive. These are mated back to strain A, and the procedure repeated for twelve or fourteen generations. Repeated backcrossing accumulates almost all the genotype of strain A in the selected mice, but the selection for resistance to the strain A tumor forces retention of histocompatibility alleles conferring resistance to the tumor. Snell reported that, under the conditions of this kind of selection, and in the great majority of instances, the difference between the original inbred strain and its essentially coisogenic mate ends up to be at a particular locus on the ninth chromosome known as the H-2 locus. The frequency with which differences at this locus turn up in this system of selection has been taken to indicate that alleles of this particular gene must play a predominant role in resistance to tumor transplantation.

The second method to be mentioned is the use of visible markers linked with histocompatibility loci, facilitating their identification and manipulation. In the late

1930's, Gorer showed that the A strain of mice had an erythrocyte antigen also present in fixed tissues that appeared to have an important relation to the fate of tumor transplants. In 1948, Gorer, Lyman, and Snell (27) found that the gene controlling this antigen was linked to a dominant gene affecting the tail, the gene "fused." The histocompatibility-erythrocyte antigen gene was at the H-2 locus already referred to. Later, Snell and his co-workers found that two other histocompatibility loci, H-1 and H-3, were linked, respectively, with the gene for albinism, *c*, in linkage group 1, and with the agouti locus, *A*, in linkage group 5. At least eighteen alleles have been identified at the H-2 locus, and at least three at H-3. Snell developed pairs or sets of coisogenic-resistant lines, permitting the study of effects of differences at these loci, probably uncomplicated by significant differences in the residual genotypes.

I shall not elaborate here the experimental procedures through which linkage with visible markers has been applied to the identification of histocompatibility differences. It should be sufficient to indicate that generations in which both the visible markers and histocompatibility are segregating can be inoculated with test tumors and data collected on the association of the marker characteristics with susceptibility or resistance to the transplant. Evidence for a close association with one of the complex of dominant tail characteristics on the ninth chromosome, for example, constitutes evidence that the histocompatibility locus segregating is H-2. The occurrence of recombination between the marker gene and the histocompatibility locus, the existence of "normal overlaps" in regard to the marker phenotype, escapes of either tumor or host in the tumor susceptibility test, and ratios grossly aberrant for unknown reasons frequently complicate interpretations from data of this sort.

Snell *et al.* (71) have now described another method for typing inbred strains of mice for histocompatibility antigens. This method depends on a system in which a known test tumor differs from a known host only at the H-3 locus. This is a "weak" difference, and under particular circumstances in a high proportion of inoculated mice the tumor grows progressively and kills its host in spite of the histocompatibility barrier. If, however, the host is first immunized by injection of tissue antigenically related to the tumor, the host is rendered capable of rejecting the inoculated tumor in a very high proportion of tests. When an unknown strain is used as the source of the immunizing tissue, this immunization will confer protection on the host if the donor material contains antigenic components related to the test tumor. The application of this system, therefore, provides a relatively rapid and sensitive basis for screening unknowns at this locus.

The years of patient and perceptive work that have been invested in the analysis of tumor transplantation genetics in the mouse have given us definitive data on specific genetic control, by individual genes, of transplantation compatibility. Even here, the number of loci thus far identified is evidently much smaller than the total number contributing tc this kind of individual variation within the species, and the extent of diversity at particular loci has only been sampled. Tumor transplantation offers opportunities for the investigation of numerous immunogenetic problems of significance in cancer research. The capacity of many tumors, in contrast to normal tissues, to override "weak" histocompatibility barriers and the wide variations in the breadth of "host range" among tumors naturally raise questions about basic aspects of the etiology and pathogenesis of cancer. Many of the studies of tumor-host relationships have a strong genetic component, related for example to variations in the number and kinds of chromosomes present in tumors, the histocompatibility factors, and their ultimate expression in the antigenic constitutions and interrelationships of hosts and tumors. The field is a large and forbidding one, on the level of our present consideration, and I shall not attempt to explore it further.

Several excellent reviews have appeared (26, 30, 41, 67).

TRANSPLANTATION OF NORMAL TISSUES

Although surgical experiences with the transplantation of normal tissues go back nearly a century, and although experimental designs directed at analyzing the genetic control of histocompatibility in normal tissues began to be applied soon after the pioneering work on tumor transplantation, much of the information available about the genetics of normal tissue transplantation derives, in a sense, from preceding tumor transplantation studies. Several references to earlier work in the transplantation of normal tissues were given in the introduction to this paper. Brief mention of later approaches to the genetics of this system, undertaken by a number of workers, will illustrate the current status of the subject. The special system of transplantation of tissues into irradiated recipients will be considered in another section of this paper.

Medawar, in 1945 (48) reported that reciprocal skin grafts among 22 rabbits were all unsuccessful and concluded that a minimum of seven independently combined antigenic differences would be required to provide this diversity. In the absence of breeding tests, of course, the genetic basis of these differences could only be speculative. The assumption of a single allelic alternative at each postulated histocompatibility locus in a population, and the further assumption that one of these alleles governs the dominant production of an antigen while its alternative is inactive and recessive, would clearly be unrealistic in terms of current knowledge of gene loci controlling antigenic specificities.

A step in a more precise direction, genetically, is illustrated by Hildemann's analysis (29) of incompatibility in transplantation of goldfish scales. The system is a remarkably convenient one, because numerous scales may be plucked from their pockets in individual fish and transferred to empty scale pockets in others, and experimental designs can thus provide complete "checkerboard" cross-testing of numbers of individuals. By restricting his studies to F_1 and F_2 generations of particular pair matings, Hildemann was able to restrict the number of possible alleles at any locus to a maximum of four (assuming that each parent may be heterozygous at the locus, and that the alleles present may all be different, i.e., $A^1/A^2 \times A^3/A^4$). Among 506 transplants, involving complete reciprocal exchanges among 23 F_1 sibs, Hildemann observed no successful transplants, although autografts were in every case accepted. In the F_2, a test of similar magnitude revealed no compatible combinations. The degree of diversity, even within a sibship and even after a generation of full-sib mating, is obviously great.

As in tumor transplantation, however, the most powerful material for analysis of genetic diversity in normal tissue transplantation is provided by inbred lines of mice. Here, crosses can be made between members of different inbred lines, and the F_2 and backcross progeny can be classified by means of skin transplants from a parent line. In this design, there is a further restriction on the kind of genetic diversity that may be present at a locus. Since the parents are members of highly inbred lines, they are presumably each homozygous at all the relevant loci. Accordingly, crosses between inbred lines may result in the introduction of only two alleles at a locus.

Again, the assumption generally made is that a graft will fail if it contains a gene controlling the production of an antigen different from any in the host. In extending calculations of "antigen number" to estimates of "gene number," the further assumptions are often made that there is a one-to-one relation between allele and antigen and that the members of an allelic series are co-dominant, as they often appear to be in the control of blood group antigens. Aside from its basis in the more thorough studies of tumor transplantation, the latter assumption rests mainly on the general experience that transplants of normal tissue from either inbred parent line to F_1 hybrids are uniformly successful, a result compatible

with an assumption that the heterozygous F_1 produces, or at least fails to respond to, all the antigens of both parents. Furthermore, skin from an F_1 hybrid is regularly rejected by either inbred parent, suggesting that at least some of the alleles inherited from either parent find expression, in the heterozygous condition, in antigenic differences recognized as "foreign" by the other parent. Although considerable experience supports these generalizations, it was only relatively recently that Eichwald and Silmser (19) observed that parent-to-F_1 skin grafts very frequently fail. The basis for failure seems in this instance to reside in antigenic differences between males and females, even within the same inbred, and presumably homozygous, lines. This observation has been confirmed and extended (66); often, but not always, male-to-female skin transplants in mice are unsuccessful. There has been some tendency to assign this phenomenon to an antigenic effect of the Y chromosome, but in the absence of definitive genetic evidence it would seem as reasonable to classify it as comparable to other secondary sexual characteristics that need not relate at all to the Y chromosome. The occurrence of some successful transplants from males to females within lines showing some degree of incompatibility in this combination can hardly be taken as evidence of genetic heterogeneity within the inbred line, but seems rather to suggest that the sex-histocompatibility effect may in these instances be weak and may or may not prevent the persistence of the transplant. We can anticipate with interest the development of more information about this provocative system.

The study by Eichwald and Silmser just referred to also illustrates another point now coming to general acceptance in the field. The experiment was designed for estimating the number of genes that might be involved in the control of skin transplant compatibility between two inbred lines of mice. Relatively early readings of the skin grafts resulted in the assumption that five or more genes were involved (20). More extended observation of the mice, however, revealed many examples of unexpected and late failure. It is evident that estimates of gene numbers in such a system will depend on the length of the period over which the success of transplants is evaluated; many "weak" histocompatibility differences come to expression only slowly. An excellent study by Barnes and Krohn (3) provides extensive quantitative data on this point. Calculating the number of genes involved in histoincompatibility for skin transplants between A and CBA strains according to the standard assumptions, and using survival for 100 days as an arbitrary criterion for compatibility, these authors concluded that certainly not less than fifteen independently segregating genes were involved. Breakdown of "successful" grafts was, however, observed as late as 180 days after grafting. On this basis, the estimate of fifteen gene differences must be considered too low. The distribution of survival times of the grafts was at variance with an assumption that quick breakdown resulted from the cumulative effect of larger numbers of histocompatibility differences between graft and host, whereas slow breakdown depended on fewer differences. As in other cases in the transplantation field, and as in such other parts of the field of immunogenetics as blood group determination, it is clear that different loci and alleles have different values in terms of effective antigenicity, and there is no simple additive relation among these, either in the evocation of an immune response or in the susceptibility of cells to the response. Another interesting point in the study by Barnes and Krohn is that the delayed reactions, once they are in train, seem to progress with reasonably constant rapidity. Potentially incompatible grafts may remain in place and apparently healthy for extended periods of time, but once their breakdown is initiated it is soon completed. What the basis may be, in the interaction of host and graft, for this long toleration of a foreign tissue followed by a relatively explosive reaction to it, remains an important problem for further research.

Prehn and Main (61) have also analyzed

histocompatibility differences between inbred strains of mice. They report calculations that the BALB/cAn and DBA/2 strains differ by approximately thirteen loci, and postulate a cumulative effect of "weak" histocompatibility differences. These workers (60) earlier raised the interesting possibility that the histocompatibility alleles were less effective in single than in double dosage, based on longer survival in one parent of F_1 tissue than of tissue from the other parent. The F_1 is, of course, heterozygous at loci for differences at which the parents are homozygous. There now seems to be considerable basis for doubt (3, 9) that this "dosage phenomenon" is general in the field of tissue transplantation. A dosage effect is encountered very commonly in blood groups.

Again as in tumor transplantation, the identification and manipulation of particular histocompatibility loci have lagged behind more general considerations of genetic diversity. It is here that the dependence on prior and pioneering work with tumors is most evident; the detection of specific loci in normal tissue transplantation is, as far as I know, entirely restricted to observations that loci identifiable in tumor transplantation studies also affect compatibility of normal tissues. Among these, the H-2 locus already referred to is again one of overwhelming prominence.

Snell's "isogenic resistant" sets of mouse strains have provided the most precise basis for the identification of particular genes with histocompatibility in normal tissue transplantation. These lines, used as co-isogenic pairs in which the residual genotypes may be assumed to be virtually identical except for a locus selected by successive generations of tumor transplantation, also display incompatibilities in skin graft exchanges (16). It turns out that a difference at the H-2 locus alone may result in rejection of skin grafts about as rapidly as do the differences at numerous loci usually prevailing between ordinary inbred strains. Strain-pair differences at only the "weak" H-3 locus permitted considerably longer survival of exchanged skin grafts—average survivals, in fact, 2 or 3 times the 13 days maximum found for differences at the H-2 locus. In contrast to the observation by Barnes and Krohn previously cited, the course of the reaction was also slower and less severe, as well as delayed in its onset, in the "weaker" antigenic difference. A difference at the H-1 locus gave highly variable graft survival times, ranging from 10 days to as long as 112 days. Variations in technique may have been partly responsible for this wide range of survival times.

As Snell pointed out, these and similar findings suggest that significantly prolonged homograft survival may be attained "with only a partial matching of the genotypes of donor and host." Applying this consideration to clinical homografting in man, one may suggest that the genetic correlations between relatives may frequently result in compatible genotypes at major histocompatibility loci, and, although minor differences may still result in the ultimate rejection of the transplant, this rejection may be considerably delayed. Lindsley[1] calculated that, with the standard assumptions of relations between gene and antigen, the probability of compatibility at any locus is higher for full sibs than between parent and offspring. This probability of course drops off rapidly as lesser degrees of relationship are considered. Nevertheless, if Hildemann's extensive experiences with scale transplantation within sibships of goldfish, even after a generation of full sib mating, may be accepted as a model of the population situation for skin transplantation as well, the probability of a compatible transplant, or even of a degree of compatibility permitting significantly prolonged survival, between sibs is so small as to discourage random trials in a search for compatible donors in a clinically important situation. What is obviously needed is some practical test basis for "transplantation-typing" large numbers of individuals. Even given such a test, however, we may well wonder whether fully compatible combinations may not be

[1] D. L. Lindsley, personal communication, 1957.

so infrequent as to prove of little practical significance.

There is, as yet, no real "genetics" of tissue transplantation in man; genetic considerations have been largely restricted to the comparison of fraternal and identical twins and to calculations of possible frequencies of successful transplants (63). It is conceivable that current studies of human tissues in tissue cultures may eventually provide tools for a satisfactory genetic analysis (59).

A remarkable aspect of the immune phenomena associated with the "second-set response" in normal tissue transplantation is its apparent specificity for the particular combination of host and donor involved in the original sensitization. A general rule cannot be stated at this time, but there have been repeated indications that, although a host sensitized by the rejection of an incompatible skin transplant will give a second-set response to a repeated transplant from the same donor or strain, only a first-set response often occurs on implantation of tissue from a different donor or strain. Perhaps the most straightforward data of this sort are reported by Barnes and Krohn (3), who found that F_2 mice from crosses between A and CBA, after rejecting A skin, gave reactions of the first-set type to subsequent grafts of CBA skin. In this experimental design, such a result need not be surprising, if it is assumed that an F_2 mouse will react to the skin from one parent only on the basis of homozygosity at particular histocompatibility loci for the alleles contributed by the other parent; i.e., only if an allele present in the test graft is lacking in the test host. A later skin graft from the other parent to this mouse would require for its reaction a completely different set of loci, those at which the mouse was homozygous for the alleles from the *first* parent. A first-set response to the new antigens introduced by the graft from the second parent might therefore be expected. This is not to say, of course, that the antigens involved are necessarily in one-to-one relation to the alleles, but only that the genetic constitution of the host must place close restrictions on the kinds of graft specificities to which he will respond. It is more surprising to find that skin grafts exchanged between unrelated individuals may also fail to sensitize for a second-set response to different donors (49). Almost anywhere else in immunogenetics, one would expect to find shared or similar antigens distributed through the population in such overlapping ways as regularly to promote second-set responses under these conditions. If they do not, in fact, regularly occur, the situation may be somewhat reminiscent of that encountered in serotype serology of ciliates (47), where the absence of cross-reactivity among different cells and their corresponding antisera appears to result from a situation in which, in spite of a diversity of genetic potential, only a single serotype may be expressed by a cell at one time. Even here, however, antigenic specificities controlled by allelic genes appear to be subject to some degree of cross-reaction (4).

TRANSPLANTATION INTO IRRADIATED RECIPIENTS

One of the most active areas of tissue transplantation research involves the injection of potentially hemopoietic tissues into irradiated recipients. No detailed discussion of the rapidly growing literature in this area can be undertaken here; suffice it to say that 3 or 4 years ago several groups of workers independently and almost simultaneously discovered that bone marrow injected into an irradiated animal becomes established as a hemopoietic transplant (22, 37, 54, 78). X-rays, at relatively high dose levels, depress the host's ability to give an immune response, destroy his hemopoietic system, and create a "vacancy" in which the injected marrow settles and multiplies. If the donor marrow is "isologous" (that is, from the same inbred line), it persists without incident. If the donor tissue is either "homologous" (from a different inbred line or a genetically different individual of the same species) or "heterologous" (from another species, as, for example, rat into

mouse), the radiation has the effect of breaking down the initial histocompatibility barriers that would be set up by a nonirradiated host, and the transplant may become established and function. A high proportion of hosts so treated survive for considerable periods after radiation doses that that would otherwise quickly be lethal to them.

Particular interest here attaches to the delayed incompatibility that develops in animals treated with homologous or heterologous hemopoietic tissues (24). These delayed reactions frequently result in the death of the host, and their basis is being ardently debated at present. The application of certain "genetic" evidence in this debate provides our reason for discussing this subject here.

The controversy revolves around the question whether the delayed reactions result from a slowly recovering immune system in the radiologically damaged host, reacting with the foreign transplant, or whether the graft may be reacting against its histoincompatible host. The latter possibility has come to general attention largely through observations by Billingham *et al.* (7) of the effects of injecting adult spleen cells into newborn animals. Such injections result in the destruction of host lymphatic tissues, in a syndrome described as "runt disease," often leading to the death of the recipient and among survivors, in a persistent tolerance to skin grafts of donor type. The injection of homologous spleen cells into irradiated adults may also result in an extensive "graft-versus-host" reaction (65). Although it is a matter of common recognition, therefore, that spleen or lymph node cells are immunologically competent to produce such a reaction, the extension of this kind of explanation to the delayed effect of foreign bone marrow in irradiated hosts depends on the assumption that this tissue also normally contains a sufficient quantity and quality of immunologically competent cells to produce a fatal graft-versus-host reaction. The case for the negative in this debate has been based almost entirely on immunologic observations (46); for example, it has become evident that there is no arbitrary x-ray dose beyond which a surviving host fails to recover, in time, its capacity to give an immunologic response. In fact, in the bone marrow experiments, the period required for host recovery in this respect is closely correlated with the time at which a delayed incompatibility reaction appears. Furthermore, pre-immunization of the host with antigenic material related to that of the prospective graft results in regular early death of the irradiated and bone marrow-treated animals, an effect suggesting the induction of a secondary response in a host-versus-graft reaction. The delayed reaction does not correlate, in a positive way, with the dose of bone marrow in the irradiated recipient, as might be expected if the bone marrow were contributing antibody-producing cells. This is in contrast to the results when spleen is used instead of bone marrow, where one expects and observes that increasing the cell dose increases the speed and extent of the reaction. Furthermore, several lines of evidence[2] now suggest that cells derived from the established bone marrow are in an unfriendly environment; they display indications of the kinds of sensitization that might be expected if a recovering host mechanism were attacking them. Nevertheless, in many instances the pervasiveness of this kind of graft and its development in overwhelming quantity seem to render it capable of withstanding, or even of suppressing, significant host responses over long periods of time. Some general term like "immunologic paralysis" may be used to describe the host's condition over this interval. The occasional long-term survivors of even heterologous bone marrow transplantation are as yet unexplained; perhaps, in some circumstances, the host may become "tolerant" to the injected material. The host, however, does not regain normal immunologic reactivity unless he succeeds in sloughing the transplant altogether (25).

The argument for a graft-versus-host

[2] R. D. Owen, and T. Makinodan, unpublished data.

basis for the delayed death pattern (33, 75, 77) also has some immunologic components, though these, perhaps like the immunologic arguments just stated for the negative, have answers more or less persuasive, depending on who is evaluating them. The most compelling point, however, is a genetic one. It is asserted that, in bone marrow experiments involving reciprocal exchanges between inbred parents and their F_1 progeny, the delayed reaction occurs with greater regularity and severity when parent marrow is injected into irradiated F_1's than in the reverse. In terms of the usual experience in tissue transplantation, this should be considered a strong indication that graft-versus-host reactions are important in this system; the F_1 graft, if it has inherited all the parental antigens, should tolerate its parental host and should therefore fail to give a delayed reaction in the parent. Similarly, a parental graft should react to antigens inherited by the F_1 host from its other parent. The reverse situation would be expected if the delayed reaction were predominantly host-versus-graft.

This system is a relatively complex one, in which a number of variables in addition to histocompatibility differences between graft and host may be important. For example, the same absolute dosages of radiation produce different physiological levels of damage in different strains and hybrids; the LD_{50} and LD_{100} levels vary markedly from one strain or hybrid to another, and one can hardly be confident that effects on the immune mechanism are necessarily completely correlated with lethality effects of a given radiation dose. Furthermore, comparisons are to be made between different inbred lines, differing in their general vigor, and with F_1 hybrids that are typically much more vigorous than their inbred parents. This difference may well extend to the cellular population comprising the injected marrow. Although it seems probable that the primary reaction in the delayed death pattern has an immunologic basis, because the reaction does not occur when isologous marrow is used, the degree of immediacy between the initial reaction and eventual death of the host is not known. It is conceivable that under some circumstances the path from initial reaction to ultimate death may be indirect, involving, for example, the depletion by an antigen-antibody reaction of substances nonspecifically involved in protection against bacterial or virus infection, and resulting in eventual death by infectious disease. Furthermore, in this complex system, the use of inadequate marrow doses may have a profound influence on the ultimate effect; studies in which this variable is not controlled or uniform may give misleading results. Under the best of circumstances, the control of this variable is subject to reservation; counts may be made of nucleated cells in the inoculum, but, while we remain in ignorance of the particular cell types that may be causally related to either the protection or the delayed reaction, we cannot be sure that any two inocula are similar, especially when the inocula to be compared come from sources as different as inbred lines and hybrids. In addition, the assay here is a matter of how many hosts die at particular times, and the establishment of valid conclusions requires adequate statistical design, sampling, and test techniques. It is also important to consider the sexes of donor and recipient. Analogy with the skin transplantation studies previously cited in this connection makes it probable that F_1 females in some combinations would give a host-versus-graft reaction to parental male tissues, in apparent contradiction to the usual genetic "laws" of transplantation. Altogether, therefore, it is still possible to doubt that F_1 marrow in parents has a real and consistent genetic advantage over parental marrow in hybrids—the genetic basis of the graft-versus-host argument. We may, however, take the argument as granted for purposes of further discussion.

Our main concern, then, is with the conclusion that the genetic "laws" of transplantation point compellingly to a graft-versus-host mechanism for the delayed reaction. Here, it seems to me, some common caution is indicated by experiences elsewhere in the

field of immunogenetics. Even if it is assumed that the antigens responsible for the delayed death pattern in the bone marrow experiment are ordinary cellular antigens comparable in their inheritance to those more thoroughly investigated in the field of red cell immunogenetics, a general assumption that an F_1 hybrid between two homozygous parents will necessarily have all the antigens of both parents and no additional antigenic specificities has been contrary to facts established and generally recognized more than 20 years ago (32). More recent studies reveal the existence of genetically independent recessive suppressors of blood group antigens (36) and of interactions between alleles resulting in the appearance of "hybrid" specificities on cells (13, 15) and the interallelic suppressions at the Rh locus, discussed during this symposium by Ceppellini. These are among a variety of lines of evidence dictating caution in assuming too simple a relation between gene and antigenic specificity, particularly in a field in which so much remains to be learned as that of the immunogenetics of tissue transplantation.

When we turn from the cellular specificities themselves to other types of antigenic materials, such as the soluble antigens of human saliva, the control of specificities by single co-dominant alleles is a subject of equally long-standing and commonly recognized reservation. The recessive block to the secretion of blood group substances, genetically independent of the ABO blood group locus itself, has been known since 1930, and additional complex interactions among alleles and among independent loci affecting the character of these secretions have been discussed by Ceppellini at this symposium. Fox and his associates have reported indications of similarly complex effects in Drosophila homogenates, and I understand that Dr. Fox recently discussed this material with reference to its possible relation to tissue transplantation genetics (23). Even the antigens of cell surfaces may sometimes relate to these complex genetic situations, as witness, for example, the sequence of independent gene-controlled reactions affecting the antigenic character of red cells in sheep (62, 73). In the bone marrow experiments, in particular, it may be important to recognize that we are dealing with the establishment of a very diverse and pervasive tissue in irradiated recipients—cells whose progeny may have a great variety of functions and products. I would be surprised if it should prove that all the potential systemic antigens relate in a one-to-one way, each to a dominant allele, even if the establishment of the transplant itself should do so.

Although the available data on the transplantation of tumors and skin, as well as of several other normal tissues, seem to point to a usually direct relation between co-dominant alleles and the significant antigens, it seems to me still possible that different antigens, subject to a different kind of genetic control, may come into play under the conditions of the delayed reaction in bone marrow transplantation. Antigens peculiar to a parent may pile up behind recessive blocks in the inbred lines that must be homozygous for recessives at numerous loci, and heterozygosity in the F_1 hybrids may release these blocks, with the result that the F_1 may well *lack* specificities found in a homozygous parent, and may *have* specificities not found in either parent. Such a consideration is recognizably speculative at this time. We cannot point to known antigens displaying the patterns of inheritance I have just postulated and important in the delayed bone marrow reaction, though we know that such antigens exist in systems such as the human salivary secretions.

At present, our best judgment would probably favor the acceptance of the "genetic" evidence from reciprocal exchanges between parents and F_1 as supporting a graft-versus-host basis for the delayed death pattern. My point is that this evidence is not as yet completely compelling, and it should be considered as contributory rather than conclusive in its bearing on the mechanism of the delayed reaction. As often happens in scientific con-

troversy, it will not be surprising if proponents of both interpretations turn out to be right, and both graft-versus-host and host-versus-graft reactions prove important.

In concluding this section, brief further reference should be made to the best-known histocompatibility locus, H-2 in the mouse. As mentioned earlier, this locus appears to be involved in the control not only of histocompatibility reactions, but of antigenic characteristics of red cells as well. In the latter connection, the locus is subject to representation as a series of symbols, representing patterns of antigen-antibody reactions between the reagents at hand and the set of related cell types controlled by alleles at the locus. The interpretation of the relation of these symbols to the structure of the antigens themselves, and ultimately to the structure of the gene, is subject to the same kind of reservations as apply to similar complexes in cattle, fowl, and man. The locus appears to be peculiarly unstable (1, 31), and modified alleles derived from hybrids have been interpreted as cross-overs among adjacent loci (2). In the absence of genetic markers on each side of the locus establishing recombination as a fact, it would seem proper to reserve judgment on the origin of these mutation-like changes; modern genetics provides possibilities other than classic recombination for such effects.

As is true elsewhere in immunogenetics, a given allele at the H-2 locus may have an effect similar to that of heterozygosity for two others (70). Gorer and his colleagues have reported several remarkable phenomena associated with this locus; for example, antigenic specificities of the red cells of one animal but not expressed on the red cells of another may be present elsewhere in his body. What may be the genetic basis of this distributional variation within the organism and what other deviations may inhere in this locus from the classic simplicity of an earlier immunogenetics remain to be revealed. At present, it seems likely that information of the greatest interest for general genetics and immunology, as well as for the more circumscribed field of the immunogenetics of tissue transplantation, will come from work in progress on the H-2 and other histocompatibility loci of the mouse.

"IMMUNOLOGICAL TOLERANCE"

The final consideration will be of the genetic aspects of a system operationally the opposite of tissue transplant rejection, a system in which specific compatibility is conferred on a host, with respect to donor tissue toward which he would otherwise develop incompatibility. Several ways of suppressing immune reactions have been applied in the field of tissue transplantation—for example, "conditioning" hosts by cortisone treatment or irradiation, or both (74), and the preinjection of derivatives of prospective donor material, which may have effects either of immunization or of enhancement of the growth of tumor grafts depending on the dose of materials used in the preinjection (68). I have recently reviewed the general subject of suppression of immune responses (56) and shall not develop it in detail here. My concern will be restricted to the particular situation described as "immunological tolerance," the specific reduction in reactivity to homologous tissue transplants conferred on an animal by the introduction, during fetal or neonatal life, of homologous tissue cells. This area, a very active one at present, traces back to experiences of experimental embryologists, who have known for many years that grafts exchanged between embryos may persist for long periods of time. In chicks, even after hatching, homologous skin grafts may be accepted and tolerated indefinitely, as in the classic "Danforth preparation," studied rather extensively during the late 1920's (17).

It is not remarkable that the young animal fails to reject foreign tissue. The immunologic machinery, a relatively late developmental acquisition, fails to function effectively in the embryonic or neonatal period in many birds and animals. But why should foreign tissues established prior to the appearance of this machinery continue to be tolerated after the animal's capacity to

give an immune response should have matured? Furthermore, as the elegant experiments of Medawar and his associates have shown (6), the tolerant animal continues to accept new transplants of the donor sort. What is the basis of this persistent and specific modification of the histocompatibility "recognition-and-response" machinery, which up until this point in this paper has been considered the more or less direct result of the animal's genetic constitution? It is on the level of the genetic control of somatic development and differentiation that the subject of immunological tolerance offers its most exciting prospects and poses its most puzzling questions.

On the level of gene numbers and the activities of particular genes in tissue incompatibility differences among individuals, the simplest description of immunological tolerance would represent this phenomenon as a straightforward resultant of the histocompatibility characteristics of host and tolerated donor. The very young individual may accept the colonization of donor tissue cells, and be thereafter a chimera of host and donor cells; this happens naturally in a high proportion of fraternal twin cattle (55) and rarely in human fraternal twins (11, 18, 53). On this basis, the tolerant individual might be regarded as the sum of its host and graft components, a new entity capable of reacting normally to grafts distinct from it, but accepting without challenge grafts identical with either of the components of the entity. The reported specificity of immunological tolerance is compatible with such a conception (8). However, a statement of this sort is of course not an explanation of the phenomenon of immunological tolerance. It is rather a restatement of the problem in terms that describe the ultimate behavior of the individual but leave unanswered the questions of why and how foreign and potentially incompatible cells introduced into the young animal may prevent the development of, or suppress, the normal histoincompatibility reactions to these cells and to others like them later introduced.

"Runt disease" and the widespread and often almost total destruction of host lymphatic tissues often observed when adult homologous spleen cells are injected into young animals (5) would surely be expected to reduce the over-all immune responsiveness of the host in nonspecific ways. Studies of the general immune responsiveness of survivors of this treatment, comparable to the studies of the immune status of irradiated bone marrow-treated mice previously cited, might be of interest in this connection. Even in the survivors of runt disease, however, a residual specificity of reaction is evident. And tolerance is conferred by tissues other than adult spleen, which do not produce detectable destruction of host lymphatic tissue or cause runt disease. This effect is therefore not a necessary concomitant of immunological tolerance.

A recent study has shown that, in contrast to identical twins, fraternal twin cattle —presumably chimeras for each other's tissues and fully tolerant to skin grafts exchanged between them—give somewhat different responses to skin grafts from their dam. Since both members of such a twin pair are chimeras for the same two types of tissues, in which the roles of host and tolerated graft are simply reversed, it seems that some components of the tissue incompatibility reactions remain unique to the host, as a function of his particular genetic constitution, regardless of the presence of a tolerated transplant. Such a situation could be expected if a role is assigned to systemic antigens produced by tissues that are not mosaic, in the fraternal twins. A model is provided by antigen J (72), which is either present on or absent from all the red cells of a chimera as a function of the host genotype only. The response of twins differing in such an antigen to maternal tissues having the antigen would be expected to be different. The implication of antigens controlled in this fashion would, however, deviate from a principle currently prevalent in the field that the antigens involved in tissue incompatibility are, in both host and graft, direct functions of the nuclear constitutions

of the cells individually concerned and are alike for all cells of the same nuclear constitution. It should be evident that other bases for the asymmetrical response of fraternal twin cattle to maternal skin grafts can be conceived; perhaps another possible explanation would be the assumption that the immune systems of the twins are not effectively mosaic but are different in the two animals and react differently to maternal antigens not present in either twin.

A field of somewhat peripheral genetic interest, though of considerable general importance, upon which the concepts of immunological tolerance impinge, relates to the relationship between mammalian fetus and mother. Since the developing fetus is doubtless characterized by antigens distinct from any in the mother, the question of why the fetus does not evoke a "homograft reaction" has led to provocative discussions, particularly by Brambell (12) and by Medawar (50). In the direction fetus-into-mother, Rh sensitization, of course, provides evidence of breakdown of the usually effective mechanism through which maternal responses to fetal antigens, and subsequent immunologic effects on the embryo, must usually be prevented or suppressed. Levine's discovery (35), that ABO group compatibility of the mother toward fetal cells favors Rh sensitization, throws a new and different light on this interaction. It suggests that quick disposal of fetal antigens, rather than indifference to them, may sometimes be involved in the maternal "tolerance" toward the fetal "homograft." In cattle, Billingham and Lampkin (10) have recently reported frequent sensitization of dams to skin grafts from their progeny, presumably as a result of normal transplacental immunization of the dam. In the other direction, there has been some suggestive evidence of at least occasional tolerance conferred on embryos by the entry of maternal cells or antigens into them (8, 57). More recently, it has appeared that x-radiation during pregnancy might promote such a process; Hasek (28) refers to a finding that rats x-rayed *in utero* are rendered specifically tolerant to their mother's skin (not to their father's), and it has also been reported that the injection of isologous embryonic liver breis intraperitoneally into pregnant female mice, after relatively low doses of radiation, promotes the growth and survival of the young (58). The latter result was interpreted as evidence that a humoral principle from the injected liver traversed the placenta and promoted recovery from radiation effects, but the possibility of the establishment in the embryos of true chimerism as a result of the transplacental passage and establishment of intact cells from the injected liver brei has not been ruled out.

Another level of genetic interest relating to immunological tolerance is the possibility that "graft adaptation" in histocompatibility characteristics may sometimes be involved in this phenomenon. Although in particular systems changes in the graft may occur (14), adaptive changes in the transplanted tissue do not appear to be a necessary condition for tolerance. The changes that do occur, in some systems, and that may result from the selection of tolerated variants or, possibly, from a "type transformation" or "serotype transformation" of graft cells (34) are of the greatest genetic interest. Nevertheless, the fact that normal spleen cells injected into a tolerant animal cause him to reject the erstwhile tolerated transplant (8) indicates that the established graft continues to elaborate its own specific antigens, which are capable of evoking an immune response in normal cells, and that the graft remains vulnerable to the effects of such responses. Similarly, the maintenance of their original antigenic type by erythrocytes derived from exchanges of erythropoietic tissue in embryonic cattle, sheep, rats, chickens, and man and the absence of any evidence of cellular diversity that might result from the exchange of "transforming" materials at the subcellular level suggest strongly that the initial lesions of immunological tolerance are in the host's recognition and response machinery, and not in a change in the transplant.

The key problem, of course, remains that

of the nature of this lesion, and to this problem there is as yet no convincing answer. We do not know enough about the normal development and biologic nature of this machinery to do more than speculate about processes that might modify it in specific ways. A vague but exciting identification of both immunity and tolerance with genes and their actions in somatic development and differentiation is subtly current in this field. Perhaps it is well that our discussion should stop with an implication of that subtle excitement, a "feeling" that tissue transplantation and tolerance may throw new light on some of the most basic and challenging problems of biology.

REFERENCES

1. ALLEN, S. L. Linkage Relations of the Genes Histocompatibility-2 and Fused Tail, Brachyury and Kinky Tail in the Mouse as Determined by Tumor Transplantation. Genetics, **40**:627–50, 1955.
2. AMOS, D. B.; GORER, P. A.; and MIKULSKA, Z. B. An Analysis of an Antigenic System in the Mouse (the *H*-2 System). Proc. Roy. Soc., London, s.B, **144**:369–89, 1955.
3. BARNES, A. D., and KROHN, P. L. The Estimation of the Number of Histocompatibility Genes Controlling the Successful Transplantation of Normal Skin in Mice. Proc. Roy. Soc., London, s.B, **146**:505–26, 1957.
4. BEALE, G. H. The Genetics of *Paramecium aurelia*. Cambridge: Cambridge University Press, 1954.
5. BILLINGHAM, R. E., and BRENT, L. A Simple Method for Inducing Tolerance of Skin Homografts in Mice. Transplantation Bull., **4**:67–71, 1957.
6. BILLINGHAM, R. E.; BRENT, L.; and MEDAWAR, P. B. Actively Acquired Tolerance of Foreign Cells. Nature, **172**:603–6, 1953.
7. ———. Acquired Tolerance of Tissue Homografts. Ann. New York Acad. Sc., **59**:409–15, 1955.
8. ———. Quantitative Studies on Tissue Transplantation Immunity. III. Actively Acquired Tolerance. Phil. Trans. Roy. Soc., London, s.B, **239**:357–414, 1956.
9. BILLINGHAM, R. E.; BRENT, L.; MEDAWAR, P. B.; and SPARROW, E. M. Quantitative Studies on Tissue Transplantation Immunity. I. The Survival Times of Skin Homografts Exchanged between Members of Different Inbred Strains of Mice. Proc. Roy. Soc., London, s.B. **143**:43–58, 1954.
10. BILLINGHAM, R. E., and LAMPKIN, G. H. Further Studies in Tissue Homotransplantation in Cattle. J. Embryol. Exper. Morphol., **5**:351–67, 1957.
11. BOOTH, P. B.; PLAUT, G.; JAMES, J. D.; IKIN, E. W.; MOORES, P.; SANGER, R.; and RACE, R. R. Blood Chimerism in a Pair of Twins. Brit. M. J., **1**:1456–58, 1957.
12. BRAMBELL, F. W. R. Immunity and Development. Adv. Science, **46**:1–11, 1955.
13. BRYAN, C. R., and MILLER, W. J. Interaction between Alleles Affecting Cellular Antigens Following a Species Cross in Columbidae. Proc. Nat. Acad. Sc., **39**: 412–16, 1953.
14. CANNON, J. A.; TERASAKI, P. I.; and LONGMIRE, W. P. Unexpected Manifestations of Induced Tolerance to Skin Homografts in the Chicken. Ann. New York Acad. Sc., **73**:862–68, 1958.
15. COHEN, C. Occurrence of Three Red Blood Cell Antigens in Rabbit as the Result of Interaction of Two Genes. Science, **123**:935–36, 1956.
16. COUNCE, S.; SMITH, P.; BARTH, R.; and SNELL, G. D. Strong and Weak Histocompatibility Gene Differences in Mice and Their Role in the Rejection of Homografts of Tumors and Skin. Ann. Surgery, **144**: 198–204, 1956.
17. DANFORTH, C. H., and FOSTER, F. Skin Transplantation as a Means of Studying Genetic and Endocrine Factors in the Fowl. J. Exper. Zool., **52**:443–70, 1929.
18. DUNSFORD, I.; BOWLEY, C. C.; HUTCHINSON, A. M.; THOMPSON, J. S.; SANGER, R.; and RACE, R. R. A Human Blood-group Chimera. Brit. M. J., **2**:81, 1953.
19. EICHWALD, E. J., and SILMSER, C. R. Communication. Transplantation Bull., **2**: 148–49, 1955.
20. EICHWALD, E. J.; SILMSER, C. R.; and CHRISTENSEN, P. F. Communication. Transplantation Bull., **2**:57, 1955.
21. FINERTY, J. C. Parabiosis in Physiological Studies. Physiol. Rev., **32**:277–302, 1952.
22. FORD, C. E.; HAMERTON, J. L.; BARNES, D. W. H.; and LOUTIT, J. F. Cytological Identification of Radiation-chimaeras. Nature, London, **177**:452–54, 1956.

23. FOX, A. S. Genetics of Tissue Specificity. Ann. New York Acad. Sc., **73**:611–34, 1958.
24. GENGOZIAN, N., and MAKINODAN, T. Antibody Response of Lethally X-irradiated Mice Treated with Rat Bone Marrow. J. Immunol., **77**:430–36, 1956.
25. GENGOZIAN, N.; MAKINODAN, T.; CONGDON, C. C.; and OWEN, R. D. The Immune Status of Long-term Survivors of Lethally X-irradiated Mice Protected with Isologous, Homologous, or Heterologous Bone Marrow. Proc. Nat. Acad. Sc., **44**:560–65, 1958.
26. GORER, P. A. Some Recent Work on Tumor Immunity. Adv. Cancer Research, **4**:149–86, 1956.
27. GORER, P. A.; LYMAN, S.; and SNELL, G. D. Studies on the Genetic and Antigenic Basis of Tumour Transplantation. Proc. Roy. Soc., London, s.B, **135**:499–505, 1948.
28. HASEK, M. Embryonic Parabiosis and Related Problems of Immunological Tolerance. Transplantation Bull., **4**:113–15, 1957.
29. HILDEMANN, W. H., and OWEN, R. D. Histocompatibility Genetics of Scale Transplantation. Transplantation Bull., **3**:132–34, 1956.
30. HOECKER, G. Genetic Mechanisms in Tissue Transplantation in the Mouse. Cold Spring Harbor Symp. Quant. Biol., **21**:355–62, 1956.
31. HOECKER, G.; COUNCE, S.; and SMITH, P. The Antigens Determined by the H-2 Locus: A Rhesus-like System in the Mouse. Proc. Nat. Acad. Sc., **40**:1040–51, 1954.
32. IRWIN, M. R., and COLE, L. J. Immunogenetic Studies of Species and of Species Hybrids in Doves, and the Separation of Species-specific Substances in the Backcross. J. Exper. Zool., **73**:85–108, 1936.
33. KOLLER, P. C. Discussion of paper by T. Makinodan. J. Cell. & Comp. Physiol., **50** (Suppl. 1):345–46, 1957.
34. KOPROWSKI, H.; THEIS, G.; and LOVE, R. Immunological Tolerance in Tumour Studies. Adaptation of Ascites Tumours to Homologous or Heterologous Hosts. Proc. Roy. Soc., London, s.B, **146**:37–58, 1956.
35. LEVINE, P. The Influence of the ABO System on Rh Hemolytic Disease. Human Biol., **30**:14–28, 1958.
36. LEVINE, P. E.; ROBINSON, E.; CELANO, M.; BRIGGS, O.; and FALKINBURG, L. Gene Interaction Resulting in Suppression of Blood Group Substance B. Blood, **10**:1100–1108, 1955.
37. LINDSLEY, D. L.; ODELL, T. T.; and TAUSCHE, F. G. Implantation of Functional Erythropoietic Elements Following Total-body Irradiation. Proc. Soc. Exper. Biol. & Med., **90**:512–15, 1955.
38. LITTLE, C. C. A Possible Mendelian Explanation for a Type of Inheritance Apparently Non-Mendelian in Nature. Science, **40**:904–6, 1914.
39. ———. Factors Influencing the Growth of a Transplantable Tumor in Mice. J. Exper. Zool., **31**:307–26, 1920.
40. ———. The Genetics of Tumor Transplantation. *In:* G. D. SNELL (ed.), Biology of the Laboratory Mouse, pp. 279–309. Philadelphia: The Blakiston Co., 1941.
41. ———. Genetics and the Cancer Problem. *In:* L. C. Dunn (ed.), Genetics in the 20th Century, pp. 431–72. New York: The Macmillan Co., 1950.
42. LITTLE, C. C., and JOHNSON, B. W. The Inheritance of Susceptibility to Implants of Splenic Tissue in Mice. Proc. Soc. Exper. Biol. & Med., **19**:163–67, 1922.
43. LITTLE, C. C., and STRONG, L. C. Genetic Studies on the Transplantation of Two Adenocarcinomata. J. Exper. Zool., **41**:93–114, 1924.
44. LITTLE, C. C., and TYZZER, E. E. Further Studies on Inheritance of Susceptibility to a Transplantable Tumor of Japanese Waltzing Mice. J. Med. Research, **33**:393–425, 1916.
45. LOEB, L., and WRIGHT, S. Transplantation and Individuality Differentials in Inbred Families of Guinea Pigs. Am. J. Path., **3**: 251–83, 1927.
46. MAKINODAN, T. Immunology of Bone Marrow Transplantation. J. Cell. & Comp. Physiol., **50** (Suppl. 1):327–51, 1957.
47. MARGOLIN, P. An Exception to the Mutual Exclusion of the Ciliary Antigens in *Paramecium aurelia*. Genetics, **41**:685–99, 1956.
48. MEDAWAR, P. B. A Second Study of the Behaviour and Fate of Skin Homografts in Rabbits. J. Anat., **79**:157–76, 1945.
49. ———. Immunity to Homologous Grafted Skin. I. The Suppression of Cell Division in Skin Grafts Transplanted to Immunized Animals. Brit. J. Exper. Path., **27**:9–14, 1946.

50. ———. Introductory Remarks. Proc. Roy. Soc., London, s.B, **146**:1–8, 1956.
51. ———. The Uniqueness of the Individual. London: Methuen & Co., 1957.
52. MITCHISON, N. A. Passive Transfer of Transplantation Immunity. Nature, **171**: 267–68, 1953.
53. NICHOLAS, J. W.; JENKINS, W. J.; and MARSH, W. L. Human Blood Chimeras. A Study of Surviving Twins. Brit. M. J., **1**: 1458–60, 1957.
54. NOWELL, P. C.; COLE, L. J.; HABERMEYER, J. G.; and ROAN, P. L. Growth and Continued Function of Rat Marrow Cells in X-irradiated Mice. Cancer Research, **16**:258–61, 1956.
55. OWEN, R. D. Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins. Science, **102**:400–402, 1945.
56. ———. Immunological Tolerance. Fed. Proc., **16**:581–91, 1957.
57. OWEN, R. D.; WOOD, H. R.; FOORD, A. G.; STURGEON, P.; and BALDWIN, L. G. Evidence for Actively Acquired Tolerance to Rh Antigens. Proc. Nat. Acad. Sc., **40**:420–24, 1954.
58. PANTELOURIS, E. M. Protection of Foetuses against Radiation Injury. Nature, **181**: 563–64, 1958.
59. PONTECORVO, G. The Genetics of Somatic Cells. J. M. Educ. (in press).
60. PREHN, R. T., and MAIN, J. M. A Comparison between Heterozygous and Homozygous Skin Homografts. J. Nat. Cancer Inst., **15**:191–94, 1954.
61. ———. Number of Mouse Histocompatibility Genes Involved in Skin Grafting from Strain BALB/cAn to Strain DBA/2. *Ibid.*, **20**:207–9, 1958.
62. RENDEL, J. Further Studies of Some Antigenic Characters of Sheep Blood Determined by Epistatic Action of Genes. Acta agr. Scandinav., **7**:224–59, 1957.
63. ROGERS, B. O. The Genetics of Skin Homotransplantation in the Human. Ann. New York Acad. Sc., **64**:741–66, 1957.
64. SCHOENE, G. Die Heteroplastische und Homoplastische Transplantationen. Berlin: J. Springer, 1912.
65. SCHWARTZ, E. E.; UPTON, A. C.; and CONGDON, C. C. A Fatal Reaction Caused by Implantation of Adult Parental Spleen Tissue in Irradiated F_1 Mice. Proc. Soc. Exper. Biol. & Med., **96**:797–800, 1957.
66. SHORT, B. F., and SOBEY, W. R. The Effect of Sex on Skin Grafts within Inbred Lines of Mice. Transplantation Bull., **4**:110–11, 1957.
67. SNELL, G. D. Methods for the Study of Histocompatibility Genes. J. Genetics, **49**: 87–103, 1948.
68. ———. Enhancement and Inhibition of the Growth of Tumor Homiotransplants by Pretreatment of the Hosts with Various Preparations of Normal and Tumor Tissue. J. Nat. Cancer Inst., **13**:719–29, 1952.
69. ———. The Enhancing Effect (or Actively Acquired Tolerance) and the Histocompatibility-2 Locus in the Mouse. *Ibid.*, **15**:665–75, 1954.
70. SNELL, G. D.; SMITH, P.; and GABRIELSON, F. Analysis of the Histocompatibility-2 Locus in the Mouse. J. Nat. Cancer Inst., **14**:457–80, 1953.
71. SNELL, G. D.; WHEELER, N.; and AARON, M. A New Method of Typing Inbred Strains of Mice for Histocompatibility Antigens. Transplantation Bull., **4**:18–21, 1957.
72. STORMONT, C. Acquisition of the J Substance by the Bovine Erythrocyte. Proc. Nat. Acad. Sc., **35**:232–37, 1949.
73. ———. An Example of a Recessive Blood Group in Sheep. Genetics, **36**:577–78, 1951.
74. TOOLAN, H. W. Growth of Human Tumors in Cortisone-treated Laboratory Animals: the Possibility of Obtaining Permanently Transplantable Human Tumors. Cancer Research, **13**:389–94, 1953.
75. TRENTIN, J. J. Induced Tolerance and 'Homologous Disease' in X-irradiated Mice Protected with Homologous Bone Marrow. Proc. Soc. Exper. Biol. & Med., **96**:139–44, 1957.
76. TYZZER, E. E. A Study of Inheritance in Mice with Reference to Their Susceptibility to Transplantable Tumors. J. Med. Research, **21**:519, 1909.
77. UPHOFF, D. E. Genetic Factors Influencing Irradiation Protection by Bone Marrow. I. The F_1 Hybrid Effect. J. Nat. Cancer Inst., **19**:123–30, 1957.
78. VOS, O.; DAVIDS, J. A. G.; WEYZEN, W. W. H.; and VAN BEKKUM, D. W. Evidence for the Cellular Hypothesis in Radiation Protection by Bone Marrow Cells. Acta physiol. pharmacol. neerl., **4**:482–86, 1956

Discussion*

W. R. SOBEY
University of Sydney, Sydney, Australia

Regarding the remarks of Dr. Owen on the failure of male to female homografts, Dr. Short and myself felt as he does that no more than a "sex effect" had been demonstrated, and references in the literature to a Y chromosome gene were premature. The sex effect is different in different inbred lines of mice. In C57, male to female grafts are rejected early, usually within 14 days, whereas in the DBA line the grafts may persist longer than 120 days. By making reciprocal crosses of such lines and grafting male to female within such crosses we were able to show that the degree of the homograft reaction was associated with the Y chromosome, i.e., a Y chromosome originating from C57 caused a rapid rejection of the graft, whereas one from DBA allowed the graft to persist.

* Following paper by Dr. Owen.

The Inheritance of "Vascular Hemophilia" A New and Interesting Problem in Human Genetics*

JOHN B. GRAHAM, M.D.†
Dept. of Pathology, University of North Carolina, Chapel Hill, N.C.

Last week I attempted to locate the earliest literary reference to a problem of blood coagulation. While reading the translation by Hartmann and Guenther of Paul Morawitz' 1905 monograph on blood coagulation,[1] I noticed that both Aristotle and Hippocrates were credited with having considered the problem. However, Aristotle was a mere 4th-century (B.C.) man and Hippocrates a 5th-century man. It occurred to me that Homer, a 10th-century poet, might provide a more ancient text containing more fundamental insights, and in the Odyssey[2] I found what I was seeking.

You recall that, when Odysseus returned from his travels, he re-asserted his authority by slaying the large group of "free-loaders" who had attached themselves to the court at Ithaca during his absence. Then, as an additional turn of the screw, he made the women of the court clean the banquet hall before he killed them for, as Homer put it, "their secret bussing and cuddling with these brave gallants". Homer describes the tidying-up scene this way.

> The women now came in with dreadful wailing and floods of tears. First they had to carry out the dead bodies, and lay them along the courtyard wall, packed close together under the gallery. Odysseus gave them directions and let them waste no time—they had to obey. Then they cleaned up the tables and seats with sponges and water. *Telemachos and the two men scraped over the floor with shovels, and the women cleared out the scrapings.*" (Italics mine.)

The scrapings, of course, were clotted blood.

When I pointed out this passage to a perceptive friend who has been impressed by the twists and turns of blood clotting doctrine and the contentiousness of the workers, he remarked that he saw profound symbolism in the fact that the earliest known literary reference links blood clotting and a shovel. "The quotation," he continued, "has a contemporary ring; it sounds like a newspaper account of charwomen cleaning an auditorium after one of the Federation's Inter-Society Sessions on Blood Coagulation."

In 1955, I attempted to analyze from the genetic standpoint a portion of the enormous literature on blood coagulation (11). It was pointed out that the blood clotting mechanism was a particularly nice physiological system to study from the genetic standpoint, since all the abnormal phenotypes have similar symptoms, i.e., poor hemostasis or hemorrhagic disease. This 1955 account was modernized somewhat and, together with a new section on the inheritance of severe multiple deficiency states, published in the proceedings of the 1956 Inter-

* The investigations of the author have been supported by research grants H-1333 and H-3140 of the National Heart Institute, Public Health Service.

† Professor of Pathology.

[1] P. Morawitz, The Chemistry of Blood Coagulation, 1905. Trans. by R. C. Hartmann and P. F. Guenther. Springfield, Illinois: Charles C Thomas, 1958.

[2] *The Odyssey*, Trans. by W. H. D. Rouse. New York: Mentor Books, 1950.

national Symposium on Hemophilia (12). Those interested in the genetic aspects of afibrinogenemia, Factor V deficiency, classic hemophilia, Christmas disease, the Stuart defect, or multiple deficiencies are urged to refer to these and other recent reviews (7, 26).

The information presently available concerning the genetic control of hemostasis cannot be summarized in a few minutes. Rather than attempting a superficial general summary, I shall try to analyze a problem concerning the antihemophilic factor (AHF), the clotting factor deficient in classic human hemophilia. This symposium seems an appropriate place for such an analysis, because it will be along genetic lines. My remarks will be concerned with the problems raised by a recently recognized hemorrhagic diathesis known as "vascular hemophilia." I hope to show that this "new" disorder exemplifies certain genetic principles which, while not "new" to geneticists, are generally not taken into consideration by blood coagulationists.

THE PROBLEM OF "VASCULAR HEMOPHILIA"

As you probably know, classic human hemophilia is conceived of as due to physiological deficiency of an essential clotting factor (AHF) and transmitted as a sex-linked recessive characteristic (6). Human hemophilia is in a class almost by itself, because there is an inherited hemorrhagic disorder in dogs which appears identical with it (13). There is no question but that the canine variety of the disorder is sex-linked and recessive (16).

It is generally recognized that the condition known as "vascular hemophilia" is inherited differently than classic hemophilia. To attempt an explanation, I shall have to weave together three strands. These strands will be referred to as "mild hemophilia," "pseudo-hemophilia," and "vascular hemophilia."

"*Mild hemophilia.*"—In the past 5 years, it has become apparent that the genetic control of the plasma AHF level is more complex than had been thought. Much new information about this matter has accumulated, largely as a result of the development of highly specific, semi-quantitative assay procedures for this clotting factor (5, 14, 22, 31).

The first clear indication that AHF level is not always controlled either by the classic hemophilia gene or by its normal allele was the report from our laboratory in 1953 of a mild form of hemophilia (15). We presumed at the time that the gene responsible for the reduction in AHF was allelic with the well recognized locus on the X chromosome. It should be emphasized that allelomorphism was a tentative suggestion. There was no direct evidence then, and there is none now, that the two forms of hemophilia are in fact allelic. Linkage data with respect to some common marker such as color blindness are not available, and no pedigrees segregating for both types of hemophilia have been described. The two types of hemophilia have in common, however, an important feature—reduction in the plasma level of the same clotting factor, AHF. The segregation of "mild hemophilia" in the human kindred described appeared consistent with incompletely recessive sex-linkage, and allelomorphism seemed to be the simplest hypothesis, as Haldane had suggested many years earlier (17). From the time allelism was first proposed, however, workers in this field have suspected that the notion was incorrect. For example, it has been difficult to understand why some of the female carriers of "mild hemophilia" have had clear reductions in plasma AHF levels while other carriers of this form of the disease and the carriers of severe classic hemophilia have not (4, 15, 27, 32).

The evidence that mild hemophilia is sex-linked is statistical and had been accepted, because sex linkage could not be rejected. The crucial test concerned the four sons of two of the five men with reduced levels of AHF. All sons were said to be clinically normal; two of the sons were available for testing and had AHF levels in the normal range, but the other two were overseas. (See Addenda for follow-up on this

point.) Also, all five of the daughters of the affected men who could be classified, either had affected sons or had reduced levels of AHF. The progeny of the thirteen females presumed to be carriers appeared equally divided between normal males, affected males, carrier females, and unclassified, but probably normal, females. The crucial test for sex linkage, of course, lay with the progeny of the affected males (Table 1). With the distribution: two normal males, two unknown males, five carrier females, and seven unknown females, a sex-linkage hypothesis could not be rejected. If "carrier" females and "affected" males are assumed to be similarly heterozygous for an autosomal dominant trait and scored "affected," an autosomal dominant hypothesis can be rejected because of the absence of affected men and normal women ($\chi^2 = 7$, $P < .01$). However, autosomal dominance cannot be rejected if as many as two of the seven unestablished females are, in reality, normal. Of course, the finding of a single affected son from an affected father rejects sex-linkage outright.

What I am trying to communicate is this: The hypothesis that "mild hemophilia" is transmitted by a gene on the X chromosome has never been considered entirely satisfactory either from the genetic or physiologic standpoint. The kindred from which the hypothesis originated was small, and a few females shifted from one cell to another, or an affected son from an affected father would cause sex-linkage to be abandoned. Interestingly, no one to my knowledge has attempted to confirm or reject this idea. It has been accepted without question and carried along in the literature for 5 years.

"Pseudohemophilia".—The term "pseudohemophilia" has probably been used at one time or another to represent every hemorrhagic disease which has been mistakenly considered identical with hemophilia. When the difference has been established, the new arrival has been, temporarily at least, designated "pseudohemophilia." I am going to use the term in a way which Humpty-Dumpty would applaud, i.e., to mean "just what I choose it to mean—neither more nor less."[3] "Pseudohemophilia" will refer to the bleeding disorder segregating in a kindred described by MacFarlane and Simpkiss in 1954 (25). This is a very interesting study for several reasons and not widely appreciated. The kindred described is fairly large; many people in it were studied by many modern methods (although the actual data were not recorded satisfactorily) and, very important, a *single* abnormality is apparently being transmitted.

Eleven of the eighteen affected members of the MacFarlane kindred, with a relatively mild bleeding diathesis and prolonged bleeding time, were tested and found normal with respect to clotting time, clot retraction, platelet numbers, morphology, and function, the various known "clotting factors," fibrinogen, thrombin generation, thromboplastin generation, prothrombin consumption, and prothrombin time. The bleeding time prolongation was transmitted by both mothers and fathers to both sons and daughters. Eight parents, abnormal by test, had 21 children who were tested. The children were distributed as: three normal males, four affected males, nine normal females, and five affected females (Table 2). This is not a significant deviation from a 1:1:1:1 ratio ($\chi^2 = 1.11$, $P > .20$), and there was male-male transmission. It appears, therefore, that "pseudohemophilia," *meaning a prolonged bleeding time without any other defect detectable by a wide variety of tests*, is transmitted in this kindred as a simple autosomal dominant.

TABLE 1

THE CHILDREN OF FIVE MEN AFFECTED WITH "MILD HEMOPHILIA"

(Graham *et al.*)

MALES			FEMALES		
Normal	Affected	Unknown	Normal	Carriers	Unknown
2	0	2	0	5	7

[3] Lewis Carroll, *Through the Looking-Glass*, p. 94. New York: Random House Edition, 1946.

"*Vascular hemophilia*".—An abstract describing an unusual hemorrhagic disorder appeared in the minutes of a national meeting in the United States in 1953 (2, 3). It described prolonged bleeding time and reduction of the plasma AHF in two patients, a man and a girl. (To my knowledge, complete case reports have never been published on these patients.) This syndrome has attracted much attention, because the time-honored axiom regarding hemophilia (or AHF deficiency) has been that the clotting time is prolonged but the bleeding time is normal.

Shortly after the first report in 1953, workers in Paris (23) reported a similar syndrome in a small French girl. In 1955, two other affected girls were reported from Holland and Canada (8, 9). The Dutch patient, in particular, had severe symptoms; her similarly affected sister had died of cerebral hemorrhage.

TABLE 2

THE CLASSIFIED CHILDREN OF EIGHT PARENTS TESTED AND FOUND AFFECTED WITH "PSEUDOHEMOPHILIA"

(MacFarlane and Simpkiss)

AFFECTED PARENTS	CHILDREN			
	Males		Females	
	Normal	Affected	Normal	Affected
6 Fathers	1	4	6	4
2 Mothers	2	0	3	1
8 Parents	3	4	9	5

Then in rapid succession in June, September, and October, 1956, seventeen other cases were reported. The mother of a girl described in June by workers from Chicago (38) had an AHF level of 75 per cent of normal, and the father had a level of 128 per cent; the Chicago workers suggested that they were describing an autosomal recessive form of AHF deficiency.

In September, a group working in New York (37) reported seven cases, four males and three females. Their material was not completely homogeneous, since six children had AHF levels of less than 1 per cent, and the seventh had 100 per cent. Also, the bleeding times of the patients ranged from a minimum one of 6–8 minutes (barely abnormal) to several greater than 15 minutes. Nevertheless, the fact that they had studied three small girls with clearly reduced AHF levels, whose fathers were not overt hemophiliacs, suggested that there might be an autosomal locus involved in regulating plasma AHF level. The New York group reported that only one parent had had a history suggestive of excessive bleeding and that studies on him were normal, thus reinforcing the recessive notion. They examined the capillaries of the bulbar conjunctivae and the nailbeds with the slit-lamp microscope and reported that the capillaries were tortuous in all seven patients. Great significance was attached to the capillary abnormality, and the name "vascular hemophilia" was suggested for the syndrome.

In October, a group working in Seattle reported five small kindreds totaling 138 persons and containing 34 individuals in whom an increased bleeding tendency was assumed from "either the history, or clinical or laboratory findings" (29). It is difficult to understand why all these patients were considered to have the same syndrome. Four persons who were extensively studied and scored as affected had bleeding times in the normal range. Four others (two of the ones with normal bleeding times and two others) had AHF levels above the lower limit of the normal range. Thus, the conclusion that one half of the persons in four of the families were affected with an identical disorder must be regarded with some skepticism. This paper was important chiefly because it reported two crucial rejections of sex linkage (father-son transmissions) as the mode of inheritance of the condition among tested individuals. Most of the extensively studied patients in this series were female (six of nine) and only three of seven patients examined had visible capillary abnormalities. Although it appeared to them that capillary tortuosity was not crucial in the pathogenesis of the condition, the authors continued to use the name "vascular hemophilia."

In 1957, four more papers on "vascular

hemophilia" appeared, three from a pair of workers in Cologne, Germany (1, 20, 21). The German workers reported two small kindreds and two sporadic cases of "vascular hemophilia." One kindred (20) showed an interesting feature. The grandfather had a bleeding time at the upper limit of normal and reduced AHF level. The father, his son, had both a clearly prolonged bleeding time and reduced AHF. Two grandsons were normal, but the grand-daughter showed a normal bleeding time and reduced AHF. The other kindred (21) showed transmission of both prolonged bleeding time and reduced AHF from two affected brothers to some of their children, one a male-male transmission.

At this point, in mid-1957, it was clear that a "new" inherited hemorrhagic diathesis had been delineated from the hazy "unclassified" category. It was being variously called "vascular hemophilia" (29, 37), "pseudohemophilia B" (38), and "angiohemophilia" (1, 21). There was general agreement that it was not inherited as a sex-linked trait and that reduction in plasma AHF and prolonged bleeding time were the key features. Clotting time and prothrombin consumption were usually normal to only slightly abnormal. Platelet counts, clot retraction, platelet thromboplastic activity, and capillary fragility were normal. There might or might not be abnormalities in the structure of the capillaries by microscopy. This seemed no longer essential.

The severity of the syndrome varied widely, ranging from mild epistaxis and easy bruising to severe hemarthrosis and even fatal hemorrhage. Clinical severity appeared to be a function of both the *length of the bleeding time* and the amount of *reduction in plasma AHF*. As might have been expected, the bleeding times varied widely between different studies, more so than did the AHF assays. Even with the assays, however, there was considerable variation within and between families.

Late in October, 1957, a very complete study appeared from Sweden (30). This study is outstanding because the material is large, six different families having been studied extensively, and uniform methods were used. As I hope to show in a few minutes, the Swedish study has provided information which allows the formulation of a genetic hypothesis reconciling "mild hemophilia," "pseudohemophilia," and "vascular hemophilia."

In the Swedish report both bleeding time and AHF level are recorded on many members of two of the six families, and AHF levels alone are given on large numbers in the other four families. The method of ascertainment in this study, which has been the same in all other kindreds reported in the literature, is important to note, because of the bias it introduces. All families have been discovered through a severely affected individual, and there have been very few other severely affected persons discovered. Widespread study of the six Swedish kindreds uncovered only three other severely affected individuals. One was a parent of the proband in Family E; another was a very distant relative of the proband in Family A, and the third was a dead sibling of the proband of Family D.

Table 3 contains data which illustrate the relationship between the bleeding times and AHF levels of the severely affected patients and the parents probably transmitting the reduction in AHF. It will be noted that the parent in every instance has a reduced level of AHF which is, nevertheless, considerably higher than that of his child. Also note that the affected children had very long bleeding times, while the parents' bleeding times were increased little if at all. These nine severely affected patients came from very small sibships which (including the patients themselves) totaled only thirteen persons, and only one of the severely affected persons (IV-7 of Family E) has had a descendant (V-15). (This last was a father-son transmission of both AHF deficiency and prolonged bleeding time, decisively disposing of any simple sex-linkage hypothesis for either trait.)

If one considers the possibility of transmission of the severe bleeding disorder as

a simple autosomal unit characteristic, the problem is difficult. It becomes that of understanding how a presumably heterozygous parent with a mild to moderate reduction in AHF and a normal bleeding time and mated to a normal person can produce a child with a much more severe reduction in AHF and much longer bleeding time. It is particularly difficult to consider the bleeding time prolongation a secondary effect of the AHF deficiency, because the classic hemophiliac with almost no plasma AHF has a normal bleeding time.

TABLE 3

RELATIONSHIP BETWEEN THE SEVERELY AFFECTED CHILD AND THE PARENT PROBABLY TRANSMITTING THE REDUCED AHF LEVEL

(Nilsson *et al.*)

	AFFECTED CHILD (ALL SEVERELY AFFECTED)				TRANSMITTING PARENT				
FAMILY	No. in pedigree	Sex	Bleeding time (min.)	Plasma AHF, per cent of normal	No. in pedigree	Sex	Bleeding time, (min.)	Plasma AHF, per cent of normal	Severity of symptoms
A	V, 6	F	>60	3	IV, 6	F	6	62	None
B	IV, 2	F	>60	4	III, 4	F	3½	35	None
	IV, 3	F	>60	4					
C	IV, 1	F	>60	6–16	III, 2	F	Normal	48	None
D	IV, 4	F	>60	5	III, 3	F	Normal	45	None
	IV, 5	F	dead, not studied						
E	V, 15	M	30	15	IV, 7	M	4–10	21	Severe
	IV, 7	M	4–10	21	III, 4	F	?	49	None
F	IV, 4	M	240	5	III, 5	F	Normal	25	None

I think insight may be obtained by comparing the tests from all the members of the two more thoroughly studied Swedish families who show either a reduction of AHF below 62 per cent or a bleeding time greater than 6 minutes. (I have chosen these discriminants, because the highest AHF level of a parent presumably transmitting the disorder in Table 3 was 62 per cent, and the upper range of normal for the bleeding time is said to be 5 minutes).

It can be seen in Table 4 that, aside from the probands and their fathers, there were seven persons from Family A and ten from Family B who had either prolonged bleeding times or reduced AHF or both. Five had prolonged bleeding times alone, ten had reduced AHF alone, and two had both.

The distribution of the abnormal values, which have been italicized in the table, suggests that, in the sibships of the transmitting parents, prolonged bleeding time and reduction in AHF have been segregating independently—i.e., the aunts and uncles of the probands have had one or the other defect but not both. This idea is bolstered by data on the children of certain of the siblings of the proband's mother in Family B. Four of these siblings with reduced AHF and normal bleeding time (III-6, III-12, III-15, III-16) had ten children. Seven of their ten children had normal AHF levels, and three had reduced AHF levels, but *all had normal bleeding times*. Unfortunately, it has not been possible to analyze the inheritance of long bleeding times alone through the children of persons with elevated bleeding times and normal AHF's. There were three suitable parents in Family A (IV-7, IV-10, and IV-14) and one in Family B (III-14), but either they had no children or their children have not been studied.

Table 5 shows that persons of both sexes with AHF levels below 62 per cent transmitted levels of less than 62 per cent to

one half of their children of both sexes. Thus, the Swedish material strongly suggests that the mildly reduced AHF level is transmitted as an autosomal dominant.

These findings tend to suggest a hypothesis more complex, but more reasonable, than any yet proposed—that the hereditary clinical syndrome "vascular hemophilia" results from the combined effects of two abnormal genes. One of them causes reduction in AHF; the other causes increase in bleeding time. The AHF locus is autosomal and dominant, as the Swedish material clearly shows, but variably expressive. The AHF reduction caused by this gene is almost never as severe as that caused by the classic hemophilia gene carried on the X chromosome, but the method of ascertainment has

TABLE 4

THE MEMBERS OF FAMILIES A AND B WITH ABNORMAL AHF LEVEL OR BLEEDING TIME

(Nilsson *et al.*)

PEDIGREE NO.	RELATIONSHIP TO PROBAND	BLEEDING TIME, (MIN.)	AHF LEVEL, PER CENT NORMAL
	Family "A"		
V, 6	Proband	*>60*	*3*
IV, 6	Mother	*6*	*62*
IV, 8	Mother's brother	*2½*	*55*
IV, 15	Mother's brother	2	*47*
IV, 7	Mother's sister	*15*	100
IV, 10	Mother's brother	*8*	88
IV, 14	Mother's sister	*7*	95
III, 4	Distant maternal relative	*>60*	*5–10*
IV, 5	Father	1	78
	Family "B"		
IV, 2	Proband	*>60*	*4*
IV, 3	Proband	*>60*	*5*
III, 4	Mother	*3½*	*35*
III, 6	Mother's brother	1	*20*
III, 12	Mother's sister	*3½*	*52*
III, 15	Mother's brother	1	*20*
III, 16	Mother's brother	*4–6½*	*21*
IV, 5	Mother's sister's daughter	*1½*	*44*
IV, 10	Mother's brother's son	*2½*	*59*
IV, 13	Mother's brother's daughter	*2½*	*36*
III, 14	Mother's sister	*7–10*	95
IV, 1	Father's sister's child	*7½*	75
III, 3	Father	3	85

With Prolonged bl. time : 5
With reduced (<62%) AHF: 10
With both : 2

Abnormal values are in italics.

TABLE 5

INHERITANCE OF REDUCED AHF LEVEL IN SIX SWEDISH FAMILIES

(Nilsson *et al.*)

AFFECTED PARENTS	CHILDREN Males Normal	Males Affected	Females Normal	Females Affected	χ^2	P (per cent)
9 Fathers	2	7	10	7	3.18	> 5
6 Mothers	2	2	3	6	.32	>50
15 Parents	4	9	13	13	1.30	>20

selected probands in whom the gene has had a particularly severe effect. As was pointed out earlier, MacFarlane's pedigree of "pseudohemophilia" indicates that simple prolongation of bleeding time may be transmitted as an autosomal dominant. MacFarlane also pointed out that the bleeding time prolongation was highly variable among affected individuals and within affected individuals at different times.

The possibility, suggested by the genetic analysis, that bleeding time and AHF level are unrelated phenomena, is also suggested by independent biochemical evidence (30). It has been shown by the Swedish workers that certain sub-fractions of Cohn's Fraction I, *without AHF activity*, will shorten the bleeding time of patients severely affected with "vascular hemophilia" *without changing the clotting time or AHF level!* This suggests that a previously unrecognized plasma factor is reduced in "vascular hemophilia" and possibly also in MacFarlane's kindred (25), causing the prolonged bleeding times.

How does this hypothesis about "vascular hemophilia" relate to "mild hemophilia" of the allegedly sex-linked variety, when it is known that bleeding times were normal in the "mild hemophilia" kindred? (I know that these bleeding times were normal, because I did them myself!) It will be recalled that the sex linkage hypothesis was based on a very small amount of information, and that a change of one or two persons into another category would have caused rejection of the hypothesis. "Vascular hemophilia," "pseudohemophilia," and "mild allelic hemophilia" can be reconciled if it is assumed:

a) That "mild hemophilia" is actually autosomally transmitted, the original report having been in error, and that in this kindred "pseudohemophilia," meaning prolonged bleeding time, was *not* segregating.

b) That "vascular hemophilia" occurs in families segregating for both "mild hemophilia" and "pseudohemophilia." The persons in whom both defects coincide and have strong effects are the ones with clinically severe "vascular hemophilia," i.e., the probands.

This hypothesis would explain two puzzling features of "vascular hemophilia," (*a*) why so few persons in a family segregating for "vascular hemophilia" are severely affected and (*b*) why an excess of females with this disease (25 females to seventeen males at last count) has been reported.

The scarcity of severely affected patients in a kindred, once ascertained, would be due to the low probability of being doubly heterozygous. Only one person in four would be expected to be doubly heterozygous in a sibship in which two rare autosomal dominants are segregating independently. It is probably also necessary that one or both of the abnormal genes have an unusually strong effect in order to produce the full-blown syndrome. (For example, the mother of the proband in Family A of the Swedish material would be scored abnormal on both counts, with a 6-minute bleeding time and 62 per cent AHF. Yet she had no symptoms.)

The excess of females in published reports is probably related to two factors. (*a*) When a male with severe bleeding symptoms and reduction in AHF is observed, he is usually labeled a "hemophiliac" and a bleeding time is not done. If his bleeding time is tested and proves to be abnormal, it is usually dismissed as an artifact. When a female with a hemorrhagic tendency and reduced AHF is observed, she becomes a research project; all tests are carried out on her, and she is correctly diagnosed. It is probable that our diagnostic files contain a sizable number of male patients with the syndrome lying unnoticed among the hemophiliacs. (*b*) The discovery of a "new" disease always makes alert observers re-examine cases previously set aside as obscure. Generally speaking, such a spurt of activity will result in the examination of more women than men, since females predominate among the obscure "unclassified" bleeders in the diagnostic file.

All the data necessary to test the two-factor thesis of "vascular hemophilia" are

not yet available. The following experiments should provide a crucial test:

1. Administration of Fraction 1, *lacking AHF activity*, to the affected patients in MacFarlane's pedigree of "pseudohemophilia" to see whether this bleeding time defect is the same as that in the Swedish material. This might also be done with the relatives of the Swedish patients with long bleeding times but normal AHF levels.

2. Re-study of the original pedigree of "mild hemophilia" to see whether exceptions to sex linkage (male-to-male transmission of AHF deficiency) can be found. (See Addenda.)

3. Determination of bleeding times of the children in the Swedish families whose parents had prolonged bleeding times but normal AHF levels.

If these three studies proved consistent with the two-factor thesis, I would consider it established as probably correct.

Now, if in "mild hemophilia" and "vascular hemophilia" the plasma AHF reduction results from an abnormal autosomal gene, what does this suggest about the control of plasma AHF level? The fact that loci on both the X chromosome and an autosome can effect a reduction in the AHF level suggests, as one possibility, that at least two loci are concerned with AHF synthesis. This may mean that AHF is synthesized in two or more steps. Such a possibility should occasion no surprise, because AHF is thought to be a large protein molecule. You will recall that AHF synthesis is optimal whenever one or more normal sex-linked alleles are present in the genome (normal hemizygote = heterozygote = normal homozygote). This suggests that the step under X chromosome control may be one in which a chemically simple but physiologically crucial thing happens to the molecule, such as the acquiring of an active site. It seems unlikely that at this step there are complex chemical reactions requiring large amounts and varieties of reactants. The reverse argument probably can be made for the autosomal locus. There are, of course, other ways to explain how AHF synthesis can be affected by two different loci. It will be very interesting to study a patient who is homozygous at the autosomal locus for AHF-deficiency, because those homozygous at the X chromosome locus are not more severely affected than the hemizygotes (16) and the heterozygotes are usually normal.

Now, the hypothesis that "vascular hemophilia" represents the summation along a final, common hemostatic pathway of double heterozygosity for two dominant autosomal genes, each of which alone has a slight effect on hemostasis, has greater generality in the field of hemorrhagic diseases then appears at first glance. There are already other similar syndromes on the horizon. For example, there is a hemorrhagic disorder present at high frequency among the Åland islanders (an isolated population living off the coast of Finland). It has been suggested recently that these people have a mild AHF deficiency coupled with a qualitative defect in platelets (19). Furthermore, reports are beginning to appear of patients with both mild PTC-deficiency (Christmas disease) and prolonged bleeding times (1, 18, 39, 40).

Many factors both plasmatic and cellular are now known to be necessary for normal hemostasis. As one abnormal phenotype after another is discovered, it is becoming apparent that these factors are under genetic control. The fact that the AHF level can be affected by mutation at more than one genetic locus can probably be extended to the other factors, known and unknown. This implies that there probably are many still unrecognized mutant genes in the population with *individually small* effects on clotting factors. Since the frequency of a gene in the population will be inversely proportional to the selective effects against it, genes with mild effects should be present at higher frequencies than genes with severe effects, such as the classic hemophilia gene. It would not be surprising, therefore, if certain other obscure hemorrhagic disorders proved to be mild double defects or a combination of severe and mild defects. It seems probable

that the specification of these disorders will require genetic analysis in addition to conventional blood clotting studies.

From the practical standpoint, a patient with hemorrhagic symptoms more pronounced than some well recognized laboratory procedure appears abnormal and should be considered exceptional and suspected of suffering from an additional unrecognized defect. The converse is also possible. A common laboratory procedure affected by many variables such as the thromboplastin generation test (4) or the partial thromboplastin time (22, 36) may show a defect more marked than clinical symptoms would suggest. In such a case one should suspect that the patient may have, in addition to his primary defect, reduction in one of the factors which affect laboratory tests but not intravascular hemostasis, such as the Hageman factor (10, 24, 28, 33–35). The fact that a syndrome is hereditary does not necessarily imply that inheritance is simple or unifactorial.

SUMMARY

1. The history of the evolvement of a "new" hemorrhagic disorder, "vascular hemophilia," is described. It is pointed out that this syndrome raises interesting problems in the interpretation of the genetic control of AHF synthesis.

2. The fact that the AHF level can be affected by abnormal genes on either the X chromosome or an autosome suggests, among other possibilities, that synthesis of the AHF molecule occurs in at least two steps.

3. It is hypothecated that "vascular hemophilia" represents double heterozygosity for a dominant autosomal gene affecting AHF synthesis and another dominant autosome affecting bleeding time. The crucial experiments with respect to this hypothesis remain to be done, and several are outlined.

4. It is suggested that others of the obscure hemorrhagic disorders may represent double heterozygosity for mild defects and that genetic analysis of the families of such patients is a very important part of the study of these disorders.

ADDENDA

1. Mr. Bennie Ward reinvestigated, in the summer of 1958, that portion of the North Carolina kindred of "mild hemophilia" crucial from the genetic standpoint, i.e., the affected male with three sons. Bleeding times on all four were determined again and found normal, and a reduced AHF level (29 per cent) was confirmed in the father. (The mother's AHF had previously been found to be 100 per cent.) Two sons had AHF levels greater than 100 per cent, while the third one, previously untested, had a level of 51 per cent. We expect to replicate these studies several more times before trying to decide whether this represents male-male transmission of "mild hemophilia."

2. Dr. J. H. Renwick of the Galton Laboratory kindly (and diplomatically) pointed out that I overlooked pleiotropism as an explanation for "vascular hemophilia." I think this possibility did not occur to me, because of the biochemical evidence that two factors are deficient in "vascular hemophilia" and of previous conditioning along the lines of the one gene-one enzyme hypothesis. If "vascular hemophilia" does, in fact, result from a single pleiotropic autosomal gene, this mutation is a very interesting one affecting two chemically different products.

The decision between these two possibilities appears to require widespread and careful studies of several large families with "vascular hemophilia." If pleiotropism is the explanation, additional severely affected patients should be found widely scattered through the kindreds. Also, one should find children with low AHF values whose parents show only prolonged bleeding times and children with prolonged bleeding times whose parents show only low AHF values. If the "two-gene" notion is correct, severely affected persons who are not close kin of a proband should be very rare. Also, parents

with one defect alone should not transmit the other.

3. It was reported at the VIIth Congress of the International Society of Hematology (Rome, Sept., 1958) by Nilsson, Blombäck, and Blomäck that plasma transfused from a "classic hemophiliac" to a "vascular hemophiliac" corrects the bleeding time without affecting the AHF level. This settles rather decisively the question whether AHF itself is related to bleeding time.

REFERENCES

1. ACHENBACH, W., and KLESPER, R. Angiohämophilie A und B. *Folio Haemat.* (new series), **1**:251–67, 1957.
2. ALEXANDER, B., and GOLDSTEIN, R. Dual Hemostatic Defect in Pseudohemophilia. J. Clin. Investigation, **32**:551, 1953.
3. BIGELOW, F. S. Serotonin Activity in Blood: Measurement in Normal Subjects and in Patients with Thrombocythemia Hemorrhagica and Hemorrhagic States. J. Lab. Clin. Med., **23**:759–73, 1954.
4. BIGGS, R., and DOUGLAS, A. S. The Thromboplastin Generation Test. J. Clin. Path., **6**:23–29, 1953.
5. BIGGS, R.; EVELING, J.; and RICHARDS, G. The Assay of Antihemophilic-Globulin Activity. Brit. J. Haematol., **1**:20–34, 1955.
6. BRINKHOUS, K. M., and GRAHAM, J. B. Hemophilia and the Hemophilioid States. Blood, **9**:254–57, 1954.
7. BRINKHOUS, K. M.; LANGDELL, R. D.; and WAGNER, R. H. Hemostatic Disorders: Hemophilia and the Hemophilioid Diseases. Ann. Rev. Med., **9**:159–90, 1958.
8. VAN CREVELD, S.; JORDAN, F. L. J.; PUNT, K.; and VEDER, H. A. Deficiency of Antihemophilic Factor in a Woman Combined with a Disturbance in Vascular Function. Acta Med. Scandinav., **151**:382–89, 1955.
9. DARTE, J. M. Defect of A-hemophilic Globulin in von Willebrand's Disease. Am. J. Dis. Child., **90**:561, 1955.
10. FRICK, P. G., and HAGEN, P. S. Severe Coagulation Defect without Hemorrhagic Symptoms Caused by a Deficiency of the Fifth Plasma Thromboplastin Component. J. Lab. & Clin. Med., **47**:592–601, 1956.
11. GRAHAM, J. B. The Biochemical Genetics of Blood Coagulation. Am. J. Human Genetics, **8**:63–79, 1956.
12. ———. Genetic Problems; Hemophilic and Allied Diseases. *In:* K. M. BRINKHOUS (ed.), Hemophilia and Hemophilioid Diseases, pp. 137–62. Chapel Hill, N.C.: University of N.C. Press, 1957.
13. GRAHAM, J. B.; BUCKWALTER, J. A.; HARTLEY, L. J.; and BRINKHOUS, K. M. Canine Hemophilia. J. Exper. Med., **90**:97–111, 1949.
14. GRAHAM, J. B.; COLLINS, D. L., JR.; GODWIN, I. D.; and BRINKHOUS, K. M. Assay of Plasma Antihemophilic Activity in Normal, Heterozygous (Hemophilia), and Prothrombinopenic Dogs. Proc. Soc. Exper. Biol. & Med., **77**:294–96, 1951.
15. GRAHAM, J. B.; MCLENDON, W. W.; and BRINKHOUS, K. M. Mild Hemophilia: an Allelic Form of the Disease. Am. J. M. Sc., **225**:46–53, 1953.
16. GRAHAM, J. B.; SWANTON, M. C.; and BRINKHOUS, K. M. Ten Years' Experience Breeding Hemophilic Dogs (in preparation).
17. HALDANE, J. B. S. The Rate of Spontaneous Mutation of a Human Gene. J. Genetics, **31**:317–26, 1935.
18. INGRAM, G. I. C. Observations in a Case of Multiple Haemostatic Defect. Brit. J. Haematol., **2**:180–93, 1956.
19. JÜRGENS, R.; LEHMANN, W.; WEGELIUS, O.; ERIKSON, A. F.; and HIEPLER, E. Über den mangel an antihämophilem Globulin (AHG, Faktor VIII) bei der Ålädischen hereditären Thrombopathie (von Willebrand-Jürgens), p. 125. VI Congress de la societe Europeene d'Hematologie, 1957.
20. KLESPER, R., and ACHENBACH, W. Über die Sogenannte Pseudohämophilie. Klin. Wchnschr., **35**:1007–13, 1957.
21. ———. Über eine Familie mit Angiohämophilie A (vaskuläre Hämophilie). Thromb. et Diath. Haem., **1**:223–33, 1957.
22. LANGDELL, R. D.; WAGNER, R. H.; and BRINKHOUS, K. M. Effect of Antihemophilic Factor on One-Stage Clotting Tests. J. Lab. & Clin. Med., **21**:637–47, 1953.
23. LARRIEU, M. J., and SOULIER, J. P. Déficit en facteur antihémophilique à chez une fille associé à un trouble du saignement. Rev. Hemat., **3**:361–70, 1953.
24. LARRIEU, M. J.; SOULIER, J. P., and CULOT, Y. Déficit en facteur Hageman. Sang, **28**: 152–59, 1957.
25. MACFARLANE, J. C. W., and SIMPKISS, M. J. The Investigation of a Large Family

with von Willebrand's Disease. Arch. Dis. Children, **29**:483–87, 1954.

26. MACFARLANE, R. G. Blood Coagulation with Particular Reference to the Early Stages. Physiol. Rev., **36**:479–502, 1956.
27. MARGOLIUS, A., JR., and RATNOFF, O. D. A Laboratory Study of the Carrier State in Classic Hemophilia. J. Clin. Investigation, **35**:1316–23, 1956.
28. ———. Observations on the Hereditary Nature of Hageman Trait. Blood, **11**:565–69, 1956.
29. MATTER, M., NEWCOMB, T. F.; MELLY, A.; and FINCH, C. A. Vascular Hemophilia: the Association of a Vascular Defect with a Deficiency of Antihemophilic Globulin. Am. J. M. Sc., **232**:421–33, 1956.
30. NILSSON, I. M.; BLOMBÄCK, M.; and VON FRANKEN, I. On an Inherited Autosomal Hemorrhagic Diathesis with Antihemophilic Globulin (AHG) Deficiency and Prolonged Bleeding Time. Acta Med. Scandinav., **159**: 35–57, 1957.
31. PITNEY, W. R. The Assay of Antihaemophilic Globulin (AHG) in Plasma. Brit. J. Haematol., **2**:250–64, 1956.
32. RAMOT, B.; ANGELOPOULOS, B.; and SINGER, K. Variable Manifestations of Plasma Thromboplastin Component Deficiency. J. Lab. & Clin. Med., **46**:80–88, 1955.
33. RAMOT, B.; SINGER, K.; HEELER, P.; and ZIMMERMAN, H. J. Hageman Factor (HF) Deficiency. Blood, **11**:745–50, 1956.
34. RATNOFF, O. D. Hageman Factor and Hageman Trait. *In:* K. M. BRINKHOUS (ed.), Hemophilia and Hemophilioid Diseases, pp. 124–29. Chapel Hill, N.C.: University of N.C. Press, 1957.
35. RATNOFF, O. D., and COLOPY, J. E. A Familial Hemorrhagic Trait Associated with a Deficiency of a Clot Promoting Fraction of Plasma. J. Clin. Investigation, **34**:602–13, 1955.
36. RODMAN, N. F., JR.; BARROW, E. M.; and GRAHAM, J. B. Diagnosis and Control of the Hemophilioid States with the Partial Thromboplastin Time (PTT) Test. Am. J. Clin. Path., **29**:525–38, 1958.
37. SCHULMAN, I.; SMITH, C. E.; ERLANDSON, M.; FORT, E.; and LEE, R. E. Vascular Hemophilia. A Familial Hemorrhagic Disease in Males and Females Characterized by Combined Antihemophilic Globulin Deficiency and Vascular Abnormality. Pediatrics, **18**:347–61, 1956.
38. SINGER, K., and RAMOT, B. Pseudohemophilia Type B. Arch. Int. Med., **97**:715–25, 1956.
39. SJØLIN, K. E., and VIDEBAEK, A. Christmas Factor Deficiency and Decreased Capillary Resistance in a Female with Haemorrhagic Diathesis. Dan. M. Bull., 85–87, 1956.
40. SOULIER, J. P., and LARRIEU, M. J. Déficit en facteur antihemophilique B avec allongement du temps de saignement. Sang, **28**: 138–41, 1957.

Panel: Selective Factors in the ABO Polymorphism

WILLIAM C. BOYD
Boston University, School of Medicine, Boston, Mass.

INTRODUCTION

Ladies and gentlemen, you see before you a reformed character. I formerly believed, and even used it as an argument for the use of blood groups in physical anthropology, that the blood group genes were selectively neutral. In taking this attitude I was doubtless influenced by certain physical anthropologists who had been arguing that human classification should be based on "non-adaptive" characters, as being the least likely to be changed by the action of evolutionary forces. I have since come to believe that there probably are no neutral genes and that classification has to be based on genes which have been affected by the forces which bring about racial differentiation, mainly mutation and selection. (In fairness it should be remarked that some of the anthropologists also changed their minds.) I am, therefore, a former believer in neutral genes who has recanted, and it accordingly gives me great pleasure to introduce this panel of papers on agencies possibly affecting blood group gene frequencies.

A Possible Example of the Action of Selection in Human Blood Groups?

WILLIAM C. BOYD
Boston University, School of Medicine, Boston Mass.

Some years ago my wife and I devised methods for determining the blood groups of mummies. The methods employed were serological and would be of interest mainly to serologists. In any case they have been fully described (3). We felt fully as skeptical as anybody else about claims, which might be considered to border almost on the fantastic, to have detected blood group antigens in material as much as 5,000 years old, and employed all the controls we could think of. Our confidence in the methods was increased by the fact that we found we could regularly determine the blood group of dried muscle taken from individuals of a known blood group, and by the fact that the results on Egyptian and American Indian mummies differed in just the way which would be expected from the present-day blood group distributions of these peoples. Our confidence has been further increased by later findings of organic material, including amino acids, which in general are less stable than blood group substances, in much older material (1). Finally, we observed that we regularly obtained the same results in such work whether we employed blood grouping reagents of human or rabbit origin.

In most American Indian mummies we found either no blood group antigen (leading to a diagnosis of group O) or antigen A, but in a few, including several from Peru (3), a "Big Bend Basket Maker" from Goat Cave, Texas (3), and two specimens excavated by Dr. W. W. Taylor (4) at Coahuila in Mexico, we apparently found antigen B.

We were not particularly surprised by these results at the time, for a number of workers had reported the presence of blood group B in American Indians, and in fact two groups of Indians, the Yámanas of Tierra del Fuego and the Carajas of the Amazon basin, had been reported to have very high frequencies of the B gene. It did not seem surprising, therefore, that some of the older inhabitants of the new world should also have possessed this gene. Since then, however, the situation has completely changed. The finding of B in the Yámanas and Carajas has been shown by retesting to be the result of gross technical error, and analyses of the available Indian data, including one such analysis made by me (2), have shown beyond any doubt that pure blood American Indians do not possess the gene B.

Now this absence of the gene B in the American Indians is in many ways the most surprising thing about them. American Indians are typically Mongoloid, and anthropologists are convinced that their ancestors came across the Bering Strait, some 20,000 to 25,000 years ago, from Asia, where blood group gene B is particularly frequent. It is thus not easy to explain why Indians do not also have B. There have been attempts to explain this, and I have suggested that it is the result of genetic drift or of an initial chance absence of B in the original immigrants. However, both of these explanations necessarily suppose that the numbers of immigrants were extremely small, smaller than linguistic, archeological, and cultural evidence makes probable, and the situation has remained something of a puzzle.

If B has in fact always been absent in

American aborigines, then our finding of B in the new world specimens must be an error, or the B must be the result of white mixture and the material cannot be very old. We are unfortunately not in a position to say anything new about the provenance of the Peruvian material we tested. Some anthropologists have doubted its antiquity. However, we do have new information about the material from Coahuila. Dr. Taylor assures me he was and is convinced that the stratigraphic evidence indicated considerable antiquity, and more recently carbon-14 dating has shown that the material is definitely pre-Columbian, some of it being in fact over 8,000 years old. The possibility of technical error remains, but examination of our original notebooks shows that the tests were conducted with, if anything, rather more than our usual care, and that each of these specimens was tested and retested until the material was used up. Therefore, although we admit in principle that any one of our determinations could be in error, we are not disposed to think that our Coahuila results were especially subject to this risk, particularly not to the extent of leading us falsely to report an antigen in two different specimens from the same site. Furthermore, if technical error were responsible, we should have to suppose that this also affected our work on the Big Bend Basket Maker specimen (3).

I should therefore like to suggest the possibility of another explanation. Let us suppose, for the sake of argument, that the ancestors of the American Indians reached this continent with blood group B, but that some selective force, due to the American environment or to the enforced new diet, or to some cause we do not understand, led to the (relatively) rapid elimination of gene B, so that the gene frequencies decreased, in the course of 20,000 years or so, from values in the neighborhood of 0.3 to 0. Such decrease would not be impossible if B were acted on by a selective agency as strong as some of those suggested by certain of the recent work, particularly if it were not opposed by any compensatory mechanism tending to preserve a balanced polymorphism in regard to the B and O genes (see previous papers in this panel).

If such a selective agency did act, it would explain why the American Indians, in spite of their Asian ancestry, today lack the gene B. It would also explain our finding of B in some American mummies, particularly in the Coahuila material, for we might well suppose that 5,000 years ago the elimination of B was still incomplete. At the same time, the possibility that B had already been largely eliminated might account for our failure to find B in most other American material, especially since most of this material was probably not so old.

It must be admitted that this suggestion is pure speculation, and that it is hard to think of any way to test it. If there is any truth in it, however, it offers a striking example of the action of natural selection in modifying one of the blood group gene frequencies in one part of the world.

One possibility, not indeed of proving this speculation, but of making it somewhat more probable, should be mentioned. According to Dr. Taylor, some tissue from the Coahuila specimens still remains, and is probably available. If retest of this material should again indicate the presence of the B antigen, I for one should then be inclined to put forward the above speculation for serious consideration as a possible explanation for the absence of B in American Indians.

REFERENCES

1. ABELSON, P. H. Some Aspects of Paleobiochemistry. Ann. N.Y. Acad. Sc., **69**:276, 1957.
2. BOYD, W. C. Blood Groups of American Indians. Am. J. Phys. Anthrop., **25**:215, 1939.
3. BOYD, W. C., and BOYD, L. G. Blood Grouping Tests on 300 Mummies. J. Immunol., **32**:307, 1937.
4. TAYLOR, W. W., and BOYD, W. C. Blood Groups of the Prehistoric Indians of Coahuila by Serological Tests of Their Mummified Remains. Year Book Am. Phil. Soc., p. 178, 1943.

Correlations of ABO Blood Groups with Peptic Ulcer, Cancer, and Other Diseases

C. A. CLARKE, M.D., F.R.C.P.*
University of Liverpool, Liverpool, England

The particular problem with which this paper is concerned is whether individuals belonging to different ABO blood groups do or do not differ in their susceptibility to certain common "adult" diseases. The question is not a new one. As long ago as 1921 Buchanan and Higley at the Mayo Clinic investigated the matter (4) and came to the conclusion that there was no association between any of the ABO groups and the diseases which they tested—and these included peptic ulcer, pernicious anemia, and various types of malignant disease.

Interest lay almost dormant for 30 years and was only renewed in 1953 when Aird and his colleagues (2) in England published a paper showing that there was a striking relationship between group A and carcinoma of the stomach in all parts of the country. Other authors have since published similar findings, although there is one large series, from Vienna, where the association has not been found. Aird and his colleagues (1) also investigated three other cancers, those of the colon and rectum, lung, and breast, and obtained negative results. In the same paper they described the biggest association of all, that between peptic ulcer and blood group O. They concluded that an individual who was group O was 35 per cent more prone to develop an ulcer than one who was not O.

Since 1954 a great deal of literature has accumulated. The association between group O and peptic ulcer has been found in many parts of the world—Boston, Iowa, Copenhagen, Oslo, and Vienna, to mention some of them, and it has also been found in Chinese, Japanese, and in Negroes. Usually the association has been greater with duodenal than with gastric ulcer, and it seems probable that there is a real difference between the two diseases. Table 1 shows the results we obtained in Liverpool (1955) and it can be seen that in our series the figures for gastric ulcer do not differ significantly from those for the controls. The controls in this and all the other series I have so far mentioned have been obtained either from grouped blood donors or from over-all grouped hospital populations, excluding those suffering from the disease being investigated. I shall refer to this again later.

TABLE 1

LIVERPOOL DATA (1955)

	O	A	B	AB
Duodenal ulcer: 860	505	263	62	30
Percentages	58.72	30.58	7.21	3.49
Gastric ulcer: 377	185	151	31	10
Percentages	49.07	40.05	8.22	2.65
Controls: 15,377	49.0	39.1	9.4	2.5

* Reader in Medicine.

In 1956 a group of workers in England showed that there was a convincing association between pernicious anemia and blood group A (7).

The next part of the story concerns the secretor character in duodenal ulcer. As is well known, some individuals secrete their ABH antigens in their body fluids and some do not. The character is inherited in a simple mendelian way, secretion being dominant to non-secretion. We thought that there might be physiological differences between those who did and those who did not secrete their antigens and that it might not be entirely fortuitous that the saliva and gastric juice

TABLE 2

SECRETION OF ABO BLOOD GROUP ANTIGENS; PERCENTAGES OF NON-SECRETORS (1956)

Duodenal ulcer: 973 patients	36.6 per cent
General population control: 849 patients	24.4 per cent
No heterogeneity for blood group or sex	
Comparison ulcers: Controls χ_1^2	31.64
$P<0.001$.	

of secretors contained particularly large quantities of these blood group substances. We therefore investigated (1956) the character in duodenal ulcer and found a highly significant association between the disease and non-secretion. Table 2 shows the details of this survey, and this part of the work was entirely carried out by Dr. R. B. McConnell. Table 3 shows the range of the diseases which have been tested for secretor character, and it will be seen that the only significantly abnormal finding is that for duodenal ulcer.

Table 4 shows the details of what we may regard as the well established associations between blood groups and adult diseases.

It appeared at first sight as though the various findings could be readily correlated. We surmised that individuals who were group O might be pouring out excess hydrochloric acid in their stomachs and therefore be liable to duodenal ulcer and only rarely develop gastric cancer. On the other hand, with group A, there was the association with gastric carcinoma and pernicious anemia, the latter always and the former often showing achlorhydria. Then there was the knowledge that the secretor substances were mucopolysaccharides and therefore in non-secretors there might be less of the protective mucoid barrier in the stomach.

The first criticism came from Penrose.[1] He thought that the findings might be due to stratification and that it was possible that there might be in the population a strain high in O and high in duodenal ulcer but with no causal connection. He said that before causality could be accepted family studies should be made, and we have investigated up to the present time nearly 400 duodenal ulcer sibships. In all the other series that have been published, the controls have been blood donors or over-all grouped hospital populations, but in our family studies it is the unaffected sibs who

TABLE 3

SECRETOR CHARACTER AND DISEASES

	No. tested	Per cent Nonsecretor
Controls	849	24.4
Duodenal ulcer	973	36.6
Gastric ulcer	132	28.0
Aphthous ulcer	156	23.7
Carc. stomach	167	19.2
Carc. cervix	220	28.3
Diabetes mellitus	318	26.1
Asthma	250	23.6

act as controls, and they cannot be criticized on the ground that they do not come from the same population as the ulcer population.

We have analysed the data by the method of Dr. C. A. B. Smith of the Galton Laboratory, London. The principle of Smith's method is to assess in each segregating family the chance of the propositus being O and then to compare the total observed results with the total expected. It must be emphasized that in this type of analysis it is only families which segregate for blood group—some of the sibs being O and some not O—that can be used, and many families there-

[1] Personal communication, 1953.

fore give no information. If we consider the simplest possible case, that of a duodenal ulcer sibship consisting of four individuals, two of whom are group O and two of group A, clearly the chance of the propositus being group O is an even one, and the expected result is therefore scored as 0.5. If, in fact, the propositus is group O, then the observed score is 1, whereas if he is group A, the observed score is zero.

Table 5 illustrates the results in 134 sibships which segregated for the O blood group. It will be seen that the observed score is slightly higher than the expected, but nowhere near significantly so. Nor is significance obtained if the propositus is paired with a sib of her or his own sex. These findings do not contradict the hypothesis that group O predisposes to ulcer, but they give no support to it.

Table 5 also shows the data analysed for secretion and nonsecretion. Again it will be seen that within families an individual who is a nonsecretor is not significantly more likely to have the ulcer than his secretor sib. If, however, the propositus is paired with a sib of the same sex, significance is obtained, but it seems doubtful from the way the work is going whether this will hold with more material.

Since I spoke about this in Copenhagen, there is some additional evidence of interest. Dr. Richard Doll of the Central Middlesex Hospital, London, has recently started a similar sibship investigation in duodenal ulcer, and he has very kindly allowed me to quote his preliminary results.[2] In 63 propositi with a total of 69 sibs he has found no association within families between group O or nonsecretion with the ulcer.

The explanation of these findings is difficult. We are loath to accept the explanation of stratification, since it seems incredible that the same stratification could occur here in the U.S.A., in England, and also in Denmark, Norway, and Portugal. We are therefore driven to consider the possibility, unlikely though it may seem in a disease such as duodenal ulcer, of a maternal factor. If mothers who are group O are more likely than mothers who are group A, B, or AB to produce children who will develop duodenal ulcer irrespective of *their* blood group, the ulcer population would be being bred from a high O strain and would give you the type of result which we have in fact obtained. Such a maternal effect might operate im-

[2] Personal communication, 1958.

TABLE 4

WELL ESTABLISHED ASSOCIATIONS WITH ABO BLOOD GROUPS*

	Duodenal ulcer	Gastric carcinoma	Pernicious anemia
Number of centers reporting	9	13	9
Number of patients analysed	8,272	6,795	1,498
Difference from controls	Group O +16.8 per cent	Group A +10.0 per cent	Group A +13.5 per cent

* From J. A. Fraser Roberts (9).

TABLE 5

ANALYSIS OF DUODENAL ULCER SIBSHIPS*

	Propositus Group O	Propositus nonsecretor
Expected	65.326	44.283
Observed	69	48
Difference	+ 3.674	+ 3.717
S.E.	± 5.577	± 4.705
P	> 0.5	> 0.4

* Method of C. A. B. Smith.

TABLE 6

FUCOSE IN SALIVA*

	No. tested	Mean fucose (μg/ml)	Standard error
O Secretor	23	93	±10.9
O Nonsecretor	23	78	± 6.5
A Secretor	23	86	± 7.5
A Nonsecretor	23	77	± 8.4
B Secretor	17	101	±12.1

* D. A. P. Evans' figures.

munologically or as a behavior difference between O and non-O women. There is something of a parallel if we consider the ABO blood groups of children with erythroblastosis due to anti-D. If in 1938, knowing nothing about rhesus, someone had investigated the ABO groups of erythroblastotic babies, he would have found that they were higher in O than the general population but that this association would not have held in sibships (see paper by Levine in this symposium).

Whether some antigen-antibody reaction occurring *in utero* could possibly cause predisposition to duodenal ulcer seems highly speculative, but any clue is worth pursuing, and we have continued to investigate the problem from both the biochemical and immunological points of view—in spite of our sibship results. The data may be summarized as follows:

1. Peebles Brown and his colleagues in Glasgow (3) have shown by means of augmented histamine test meals that individuals who are group O do not have potentially more hydrochloric acid in their stomachs than those who are not O.

2. Morgan (8) has pointed out that the nonsecretors of ABH are usually secretors of Lewis, and therefore, in terms of total blood group mucopolysaccharide in the stomach, there will be nothing to choose between the secretors and nonsecretors of ABH. One of our team, Dr. Price Evans, is investigating this matter at the moment. The sugar fucose provides a very good index of blood group activity, and Evans has found in his results thus far that there is no significant difference in the amount of fucose in the saliva of secretors and nonsecretors of ABH—in other words, in the nonsecretors of ABH the fucose is coming from the secretion of Lewis (Table 6). Before leaving this point, I should mention that about 8 per cent of the population are known to be nonsecretors of both ABH and Lewis. We wondered therefore, whether duodenal ulcer patients might show an excess of this type of individual, but we have not found this to be so.

3. If the association between duodenal ulcer and nonsecretion is due to a direct effect of the secretor gene, then there might be quantitative differences in antigen titer in the saliva of individuals who are secretors compared with their unaffected secretor sibs—we might expect a lower titer, i.e., less antigen in those who have the ulcer. As far as we have gone (about 70 sib pairs) there is no significant difference in titer between the saliva of the individual with the ulcer and that of the sib without. If this proves to be our final conclusion it will be strong evidence against the possibility that the association between nonsecretion and duodenal ulcer is due to a direct effect of the secretor gene.

4. Coombs has shown, by means of the mixed agglutination technique, that the ABH antigens are present not only on red cells but on many types of surface epithelium as well. Coombs found that antigens were present regardless of whether the individual was a secretor or nonsecretor. We felt that it would be worth while investigating this matter in relation to suspensions of duodenal cells, and one of our team, Dr. Frances Selsnick, an American citizen, is carrying out this work.[3] She is using fluorescein-labeled antibodies as well as Coombs' technique, and, although the results are only tentative, it rather looks as though in the case of duodenal cells the nonsecretors do *not* have antigens on their striated borders, whereas the secretors do. If this proves to be our final view, it would encourage us to explore further the possibility that there might be immunological factors at work in the production of duodenal ulcer. We did at one time postulate that ABH agglutinins in plants might be playing a part—many of the Leguminosae have anti-H or anti-A substances, and the possibility that substances such as soya beans which are used as improvers could be toxic because of their antibody content crossed our minds—but it has been shown that most of these plant antibodies are destroyed by boiling, and therefore this hypothesis rather deteriorates.

[3] Personal communication, 1958.

CONCLUSION

The whole problem, therefore, of the relationship of duodenal ulcer to blood group O remains unsolved. The association does not show up in families, and the biochemical data do not show how the association, if a true one, is exerting its effects. An immunological reaction remains a possible explanation, and we think the blood groups of mothers who have borne children who develop duodenal ulcer are worth investigating.

Nevertheless, the evidence from all parts of the world regarding the association between group O and ulcer, when blood donors are used as controls, is very strong. It is possible that, for some reason, the effect of the O gene is diluted in families because of other genes which predispose to ulcer, and these may be more in evidence in ulcer sibships than in the general population.

ACKNOWLEDGMENTS

We are grateful to the United Liverpool Hospitals Research Committee under the chairmanship of Lord Cohen of Birkenhead and to the Nuffield Foundation for their generous support.

REFERENCES

1. AIRD, I.; BENTALL, H. H.; MEHIGAN, J. A.; and ROBERTS, J. A. F. The Blood Groups in Relation to Peptic Ulceration and Carcinoma of Colon, Rectum, Breast and Bronchus. An Association between the ABO Groups and Peptic Ulceration. Brit. M. J., 2:315, 1954.
2. AIRD, I.; BENTALL, H. H.; and ROBERTS, J. A. F. A Relationship between Cancer of Stomach and the ABO Group. Brit. M. J., 1:799, 1953.
3. BROWN, D. A. P.; MELROSE, A. G.; and WALLACE, J. The Blood Groups in Peptic Ulceration. Brit. M. J., 2:135, 1956.
4. BUCHANAN, J. A., and HIGLEY, E. T. The Relationship of Blood Groups to Disease. Brit. J. Exper. Path., 2:247, 1921.
5. CLARKE, C. A.; COWAN, W. K.; EDWARDS, J. W.; HOWEL-EVANS, A. W.; MCCONNELL, R. B.; WOODROW, J. C.; and SHEPPARD, P. M. The Relationship of the ABO Blood Groups to Duodenal and Gastric Ulceration. Brit. M. J., 2:643, 1955.
6. CLARKE, C. A.; EDWARDS, J. W.; HADDOCK, D. R. W.; HOWEL-EVANS, A. W.; MCCONNELL, R. B.; and SHEPPARD, P. M. ABO Blood Groups and Secretor Character in Duodenal Ulcer. Population and Sibship Studies. Brit. M. J., 2:725, 1956.
7. Collective Series from a Number of Centres. Association between Blood Group A and Pernicious Anaemia. Brit. M. J., 2:723, 1956.
8. MORGAN, W. T. J., quoted by Levine. *In:* MCCONNELL, R. B. & SHEPPARD, P. M., Discussion on the Secretor Character and Disease. Acta genet., 6:579, 1956/57.
9. ROBERTS, J. A. F. Blood Groups and Susceptibility to Disease. Brit. J. Prev. Soc. Med., 2:107, 1957.

Selection in ABO Polymorphism in Japanese Populations

EI MATSUNAGA
Department of Legal Medicine, Sapporo Medical College, Sapporo, Japan

In order to study selection in ABO polymorphism, it is necessary, as the first step, to collect evidence from as many diverse sources as possible. As the second step, an estimation must be made of the selective forces on certain genotypes at different ontogenetic stages. Particular attention must be paid to whether a counterbalancing agent is operating, since it is thought that a constant gene frequency or equilibrium is usually maintained in natural populations. Table 1 summarizes some criteria for recognition of selection in relation to the ABO blood groups at different developmental stages. For clinical interest, selection at a very early stage may perhaps be of less interest than if grown persons of certain genotypes are more susceptible to a malignant disease. From the viewpoint of genetics, however, equal attention must be paid to selection at any stage as long as it is affecting an individual in such a way that, compared with those of the other genotypes, he cannot contribute proportionately to the gene content of the population in the following generation. In this paper, I would like to discuss the general topic using our data from Japanese populations but comparing them with those from American whites.

TABLE 1

CRITERIA FOR RECOGNITION OF SELECTION IN ABO BLOOD GROUPS AT DIFFERENT STAGES

Stages	Genotypically conditioned differences in
Time of fertilization	Fertility of parent, the sex ratio in the children
Intra-uterine	Occurrence of spontaneous abortions
Peri-natal	Occurrence of stillbirth and hemolytic disease
Post-natal and adult life	Resistance or susceptibility to diseases, longevity

The discovery by Levine and his coworkers (11) that hemolytic disease of the newborn is caused by maternal-fetal isoimmunization in the Rh blood group system has made a new epoch in the field of blood group serology, since it has established beyond doubt that the blood groups do have different selective values. This recognition stimulated a search for such selection in other blood group systems, and many reports have appeared pointing out that not only hemolytic disease but also spontaneous abortions can be caused by maternal-fetal ABO incompatibility. The term incompatibility is applied to those instances when the mother is lacking a dominant blood factor that is present in the fetus. In ABO blood groups, the following instances are incompatible: mother O and fetus A or B, mother A and fetus B or AB, and mother B and fetus A or AB. These matings are in contrast to those compatible matings in which the blood factors of the child and of the mother are identical or in which the mother carries the dominant factor.

A direct approach to the estimation of the extent of this selection could be made by family investigations in which exact reproductive differences in reciprocal mating

types are examined. During the period of from 1953 to 1954 an extensive field study was carried out in our laboratory on 1429 families of laborers from mining companies in two mining town areas in Hokkaido, namely, Ashibetsu and Kohnomae. From each couple blood specimens were collected for routine tests of the blood groups of ABO and MN systems. Examination of the $Rh_0(D)$ antigen was omitted, because the incidence of Rh_0 positive persons is assumed to be more than 99 per cent in the Japanese population, and it was considered that, for comparison of fertility between reciprocal mating types with respect to the Rh system, a much larger body of data would be necessary. Careful marital histories were taken, particular attention was paid to the number of living children, and all pregnancies including spontaneous abortions were recorded. It was found that at the time of this investigation there was scarcely any effective control over family size in the population samples of these localities.

Since the distributions of each blood group and parental combination showed no sign of heterogeneity between the two areas in both ABO and MN systems, the data were pooled for analysis of differential fertility in reciprocal mating types. In Tables 2 and 3 are presented the summaries of the results of the comparison between two major mating groups, i.e., compatible and incompatible. There was no indication for selection due to incompatibility in the MN system, whereas in ABO blood groups a highly significant difference was demonstrated between the two mating groups in two respects. In the incompatible mating group, the frequencies of abortions and of childless couples were distinctly higher than in the compatible mating group. The mean number of pregnancies per woman did not show a significant difference, although the frequency of infertile couples was higher in the incompatible group than in the compatible one, the difference being significant at the 5 per cent level. This may be explained by the possibility that selection might be at work at the earliest period of pregnancy under conditions unknown to

TABLE 2

FERTILITY OF COMPATIBLE AND INCOMPATIBLE MATING GROUPS IN MN BLOOD TYPES

	Compatible	Incompatible
No. matings	885	544
Proportion of couples as yet infertile	9.83 per cent	9.38 per cent
Mean no. pregnancies (per woman)	3.210 ± 0.077	3.173 ± 0.094
Proportion of abortions (per pregnancy)	12.28 per cent	12.69 per cent
Proportion of childless couples	13.56 per cent	13.24 per cent
Mean no. living children (per woman)	2.403 ± 0.084	2.430 ± 0.077
Mean no. living children (per pregnancy)	0.749	0.766

TABLE 3

DIFFERENTIAL FERTILITY BETWEEN COMPATIBLE AND INCOMPATIBLE MATING GROUPS IN ABO BLOOD GROUPS

	Compatible	Incompatible	Test for significance
No. matings	812	617	
Proportion of couples as yet infertile	8.13 per cent	11.67 per cent	$0.05>P>0.02$
Mean no. pregnancies (per woman)	3.250 ± 0.080	3.125 ± 0.091	$0.30>P>0.10$
Proportion of abortions (per pregnancy)	10.34 per cent	15.30 per cent	$P<0.001$
Proportion of childless couples	9.85 per cent	18.15 per cent	$P<0.001$
Mean no. living children (per woman)	2.596 ± 0.064	2.173 ± 0.070	$P<0.001$
Mean no. living children (per pregnancy)	0.799	0.696	

the mother. Further, as expected, a highly significant reduction in the mean number of living children per woman has been demonstrated in the incompatible mating group compared with that in the compatible mating group. Under the assumption that fertilization occurred with equal frequency in both mating groups, it was calculated that the mortality rate of those children incompatible with the mothers, whether the cause of death be abortion, stillbirth, or hemolytic disease, amounts to 21 per cent in this population.

The magnitude of this powerful selection pressure is not very surprising when we consider that the intra-uterine and perinatal period is the most critical one from the point of view of natural selection. To compare the present results of a Japanese population with those of Europeans or American whites, it is necessary to consider both genetic and environmental factors influencing the fetal and neonatal morbidity. The incidence of maternal-fetal incompatibility in any blood group system depends on the genetic composition of the population. Table 4 illustrates the comparison of the occurrence of hemolytic disease due to incompatibility both in ABO and Rh systems in American whites and Japanese with special reference to the incidence of the incompatible matings in these populations. The frequency of Rh incompatible matings amounts to about 13 per cent in American whites, while it is only about 0.5 per cent in Japanese. In accordance with these figures, the incidence of hemolytic disease by Rh incompatibility in Japanese is much lower than that in American whites. On the other hand, the incidence of ABO incompatible matings is about 44 per cent in Japanese, whereas in American whites it is about 36 per cent. Corresponding to this difference, the occurrence of neonatal deaths by ABO incompatibility is higher in Japanese than in American whites.

In connection with the above differences in the genetic composition of the two populations, it should be taken into consideration that there is an interaction between Rh incompatibility and ABO incompatibility. Levine (9) drew attention to the fact that mothers of children with hemolytic disease due to Rh isoimmunization were more often compatibly mated in regard to ABO system than were unselected women. This finding was substantiated by later workers on this field, and it has been explained by assuming that ABO incompatibility protects from Rh isoimmunization in the case when the pregnancy is incompatible for both the Rh and ABO systems (Race and Sanger [18]). In a study of a Swedish population by Grubb and Sjöstedt (5), it was found that in a series of marriages with two or more pregnancies terminating in intra-uterine death for unknown reasons the frequency of ABO incompatible matings was about 50 per cent in the mating class Rh-positive × Rh-positive, while in those involving at least one Rh-negative partner only about 20 per cent of the matings were ABO incompatible, and the discrepancies were statistically significant. Therefore, in

TABLE 4

INCIDENCE OF HEMOLYTIC DISEASE OF THE NEWBORN IN AMERICAN WHITES AND JAPANESE

POPULATION	FREQUENCY OF INCOMPATIBLE MATINGS WITH RESPECT TO ABO system (per cent)	FREQUENCY OF INCOMPATIBLE MATINGS WITH RESPECT TO Rh system (per cent)	INCIDENCE OF HEMOLYTIC DISEASES IN FULL-TERM PREGNANCIES DUE TO ABO incompatibility	INCIDENCE OF HEMOLYTIC DISEASES IN FULL-TERM PREGNANCIES DUE TO Rh incompatibility
American Whites	36	13	1:1500*	1:150*
Japanese	44	0.5	1:200†	1:6600~1:16000†

* After Levine (10).
† After Kihara (8).

a population such as Japanese where almost all matings are of Rh-positive × Rh-positive, it would be not so difficult to detect the effect of the selection due to ABO incompatibility, while in European populations or American whites the over-all effect of such selection could perhaps be reduced to a certain extent by the action of Rh incompatibility.

Here, it may be worth while to call attention to the fact that infant death from ABO incompatibility could begin at the first pregnancy in contrast to cases caused by Rh incompatibility where the mother will usually be more intensively sensitized with succeeding pregnancies. Table 5 represents the distribution of living children in the compatible and incompatible mating groups in our data. The frequencies of childless couples, as has been stated, differ significantly between the two mating groups, but the frequencies of those couples with one to five living children, respectively, show no significant differences, while the frequency of couples with more than six children is about 7 per cent in the compatible matings—and this figure is more than twice that in the incompatible one, the difference being again significant. Childless couples may be the result of ABO incompatibility, while one-child sterility might be produced by Rh incompatibility. The conceivable explanations for this finding may be made in the following ways: from the frequent fetal deaths caused by ABO incompatibility, it is likely that the antibody-producing power of the A and B antigens is much stronger than that of Rh, so that isoimmunization in the former case is sufficient to cause infant death in the first pregnancy. Moreover, it is possible that the normal anti-A or anti-B in mother's serum would affect the fetus early in pregnancy before the mother has been sensitized. Evidence for this hypothesis is, however, still lacking, and further research is needed.

So far we have discussed the subject from genetical and serological points of view. It is evident, however, that incompatibility is a necessary condition for such selection but not a sufficient one. Since incompatibility does not always cause abortions or hemolytic disease, there must be certain yet unknown factors which, when added to the incompatibility, cause the diseases. Apart from these factors, there are several agents affecting the magnitude of this selection. Reed (19) suggested that pre-natal selection can often be demonstrated when the mean parity exceeds two but that the degree of selection may vary with parity. As Glass (4) demonstrated in American whites regarding Rh blood group system, it can be expected that, in a population where effective control over family size exists and a pattern of very small size of family has been established, the differential fertility between ABO compatible and incompatible matings may be changed even in the opposite direction, owing to the so-called overcompensation. Moreover, general living conditions, especially those concern-

TABLE 5

DISTRIBUTION OF LIVING CHILDREN IN COMPATIBLE AND INCOMPATIBLE MATINGS

No. children	Compatible matings		Incompatible matings		χ^2 value with Yate's correction
	No.	Per cent	No.	Per cent	
0	80	9.85	112	18.15	20.06 ($P<0.001$)
1	161	19.83	130	21.07	0.16
2	198	24.38	147	23.82	0.02
3	170	20.94	102	16.53	2.78
4	84	10.34	63	10.21	0.01
5	62	7.64	43	6.97	0.12
6~10	57	7.02	20	3.24	8.17 ($P<0.01$)
Totals:	812	100.00	617	99.99	31.32 ($P<0.01$)

ing the health of the population studied, have an appreciable effect. The frequency of stillbirths including spontaneous abortions occurring after the fourth month of pregnancy that have been registered legally in the City of Sapporo in Hokkaido from 1949 to 1953 amounts to 14.5 per cent of both livebirths and stillbirths (Inouye [6]). In the population of the mining town areas investigated by us, the living conditions seemed to be much worse than in the city of Sapporo. These agents must have influenced to a large extent the magnitude of selection by ABO incompatibility in that population. However, I would like to note here that the situations are of course quite different according to locality and seem to be rapidly changing in Japan during the last few years.

Going back to the discussion of the subject from the viewpoint of population genetics, the action of selection due to maternal-fetal incompatibility is directed against children of heterozygous genotypes. The selection must work, therefore, to depress the gene ratio of that allele which is less common in the population. In the case of ABO polymorphism, it follows that, for the Japanese population as well as for populations in most parts of the world, selection would work so as to decrease the gene frequency of A and B and to increase the frequency of O. As a result, the gene ratio would no more be balanced and it would change from generation to generation until the frequencies of both dominant genes would be maintained at very low level by recurrent mutation. In view of the present higher frequencies of these alleles in human populations, it should be surmised that other selective forces must be operating in order to compensate the losses of the heterozygotes or the losses of A and B genes.

According to Fisher's theory for maintenance of a balanced polymorphism, a stable equilibrium of gene frequencies depends on opposed forces and can be produced (*a*) when a heterozygote has a selective advantage over both homozygotes or (*b*) in certain conditions when there is an interaction between two or more groups of alleles in such a way as to alter one another's selective values. An unstable equilibrium is produced, on the contrary, when a heterozygote is at a disadvantage compared with both homozygotes. These general principles can now be applied to the consideration of the possible compensatory mechanism for the maintenance of genetic equilibrium in ABO polymorphism. The first attempt to seek for such compensatory mechanism should be made, therefore, by examination whether in ABO blood groups heterozygotes may have selective advantage over homozygotes. Heterozygous individuals may have larger fertility or viability than homozygous individuals in post-natal life. It is also conceivable that compensating selection may be operating during the pre-natal period in such a way that heterozygous fetuses may have an advantage over homozygous fetuses when there is no incompatibility.

In the above data of the mining town areas there was an indication that group O fathers may be less fertile than heterozygotes. Within compatible mating groups, the mean number of pregnancies and of living children in those matings with group O fathers was significantly lower than those in the matings in which neither parent belongs to O (Table 6). Since the frequencies of abortions are almost equal in both mating subgroups, the difference in the mean number of children is obviously due to the difference in the mean number of pregnancies. The genotypes of the children from the matings, in which the father belongs to O, are AO, BO, and OO, while from those matings in which neither parent belongs to O, children of AA, BB, and AB genotypes can be born, in addition to AO, BO, and OO. The proportion of these two mating subgroups is 54:46, so that this differential fertility might be of sufficient magnitude to compensate for the losses of A and B genes eliminated through incompatibility. It is necessary, however, to test whether compensation of this form can restore the genetic equilibrium, whenever it is destroyed by incompatibility. According to a personal

communication from Dr. Kimura, who has recently analyzed this point mathematically, it was proved that, assuming the counterbalance between the effect of decreased fertility of O father and that of incompatibility, an equilibrium is reached at certain gene frequencies; but it is not a stable one. Compensation of such a form cannot lead to a balanced polymorphism.

Another form of compensation by heterosis may now be put forward in the following model; assuming only pre-natal selection, a heterozygous fetus of certain genotypes, say AO, has a disadvantage of i compared with homozygotes when pregnancy is incompatible, while it has a selective advantage of h when the pregnancy is compatible. In a random mating population, the frequencies of AO fetuses that are compatible and incompatible with the mothers are $\overline{AO}_{comp}$ and $\overline{AO}_{incomp}$, respectively. Then the following equation formula should be held for a balance of gene ratio:

$$h \cdot \overline{AO}_{comp} = i \cdot \overline{AO}_{incomp} .$$

If the value of $h \cdot \overline{AO}_{comp}$ is larger than that of $i \cdot \overline{AO}_{incomp}$, it would presumably lead to a stable equilibrium, whereas it would be changed to an unstable one if the former value were smaller than the latter. Since the heterozygotes of both AO and BO cannot, unfortunately, be recognized serologically on a wide scale, it would be difficult to collect data to test this hypothesis. However, regarding the AB heterozygote, a direct approach to the analysis of this point can be made. The frequency of AB in Japanese population is about 10 per cent, so that it is much higher than that in Europeans. The proportion of those AB children incompatible with the mother in the total AB is exceedingly high; in Japanese population approximately 78 per cent of AB fetuses are incompatible with the mother. If the selection pressure due to incompatibility is of a magnitude of 0.21, as calculated in the population of the mining town areas, then the value of h, calculated from the above formula, should be 0.74 for a neutral equilibrium. On the other hand, the relative fitness in viability of AB heterozygotes compared with AA and BB homozygotes can be calculated when we obtain sufficient data from a homogeneous population regarding the number of children of different genotypes born from AB × AB matings. From the literature of Japanese family investigations which have been carried out on more than three thousand matings at the Department of Legal Medicine of both Kanazawa Medical College and Tokyo University directed by Dr. Furuhata, 33 such matings were collected (Table 7). Of 83 children born, eleven were AA, eighteen BB, and 54 AB. The AB:(AA + BB) ratio was 1.86, which indicated a significant excess of the AB children. The observed value of h amounts to 0.86, which is higher than that value for the equilibrium. The differ-

TABLE 6

DIFFERENTIAL FERTILITY BETWEEN TWO SUBGROUPS OF COMPATIBLE MATING GROUPS

	Mother Father A×A B×B AB×AB AB×A AB×B	Mother Father A×O B×O AB×O O×O	Test for significance
No. matings	373 (46 per cent)	439 (54 per cent)	
Proportion of couples as yet infertile	7.51 per cent	8.66 per cent	0.70>P>0.50
Mean no. pregnancies (per woman)	3.472±0.125	3.062±0.101	0.02>P>0.01
Proportion of abortions (per pregnancy)	10.19 per cent	10.49 per cent	
Proportion of childless couples	9.92 per cent	9.79 per cent	
Mean no. living children (per woman)	2.743±0.101	2.472±0.082	0.05>P>0.02
Mean no. living children (per pregnancy)	0.790	0.807	

ence was not significant, but it suggests that the compensation may probably be leading in this way to a stable equilibrium.

From what has been said, it will be clear that the counterbalancing selection against that by incompatibility is most probably operating in a sufficient magnitude through the mechanism of heterosis. On the other hand, there is some evidence for compensatory mechanism through a selective interaction between ABO and other blood group systems. As has been mentioned, the interaction of ABO and Rh incompatibility has been established, and the same thing is very probably happening in many other blood group systems which can cause hemolytic disease or abortions by the same mechanism of isoimmunization. As a result of such interaction, selection against heterozygotes in a certain blood group system can be at the same time selection for heterozygotes in another system. As Woolf (24) pointed out, ABO incompatibility is working as a protection against hemolytic disease due to Rh incompatibility, so that heterozygotes are standing at a selective advantage over homozygotes when the pregnancy is simultaneously incompatible regarding Rh system. In European populations or American whites, where the Rh-negative persons are at relatively high frequency, it will be especially interesting to see whether the compensation of this form is of sufficient magnitude to be a significant factor in producing a stable equilibrium in the ABO polymorphism.

So far we have discussed the selection operating in pre-natal and early life in connection with the strong natural selection caused by maternal-fetal incompatibility. During the last few years, evidence is accumulating from different parts of the world for associations between blood groups and a variety of adult diseases. Among these, the most overwhelming is the evidence for association with three diseases, i.e., duodenal ulcer, gastric ulcer, and carcinoma of the stomach. In the first two, persons of group O are at a disadvantage; in the last one, persons of group A. Here I may quote the result of preliminary nature of recent investigations being carried out by Dr. Uetake and others in Tokyo, where a large body of data can easily be collected. The data concern only peptic ulcer and gastric carcinoma, which were obtained from the records of the Hospital of Cancer Research Institute covering the period 1946 to 1956. Diagnostic criteria depend in most cases on histological examinations but in a few cases on roentgenological or other clinical findings. The data were evaluated by comparing the observed blood group frequencies with those of about 2000 pupils of primary and middle schools. The statistical significance of the differences in blood group frequencies occurring between the patients and controls was tested by the χ^2 method.

TABLE 7

FREQUENCY OF AB CHILDREN FROM AB × AB MATINGS

INVESTIGATOR	NO. MATINGS	NO. CHILDREN AA	BB	AB	Totals
Furuhata (1932)	14	4	7	22	33
Sugishita (1935)	2		1	3	4
Suzuki (1936)	1		1	2	3
Imamura (1937)	2		1	4	5
Hayashida (1944)	3		2	4	6
Arai (1944)	2		2	4	6
Kaneda (1951)	1			3	3
Matsunaga (1954)	8	7	4	12	23
Totals	33	11	18	54	83

$\frac{AB}{AB + BB} = 1.86$; χ^2 value with Yate's correction = 6.94, $P < 0.01$.

In Table 8 are illustrated the basic data showing the results of the O:A:B:AB and O:A comparisons. No significant difference was found between sexes in any series of the data. In consistence with the results reported from the other parts of the world, a significant excess of O group in duodenal ulcer and of A group in gastric carcinoma has been demonstrated. There was an indication, though not significant, for the excess of O group in gastric ulcer. The associations seem to be causal rather than due to population stratification, since the same tendencies have been observed in different populations. However, for the purpose of discussing the genetic consequences of selection resulting from these adult diseases, available data are still too scarce.

TABLE 8

JAPANESE DATA (TOKYO) FOR ASSOCIATIONS BETWEEN BLOOD GROUPS, PEPTIC ULCER, AND GASTRIC CARCINOMA

(After Uetake, Tanaka, and Fujii, [22])

PATIENTS	BLOOD GROUPS O	A	B	AB	PATIENTS VS. CONTROLS PROBABILITY O:A:B:AB	O:A
Peptic ulcer	260	240	141	54	0.02>P>0.01	P<0.01
695	(37.4%)	(34.5%)	(20.3%)	(7.8%)		
Duodenal	71	50	29	13	0.02>P>0.01	P<0.01
163	(43.6%)	(30.7%)	(17.8%)	(8.0%)		
Gastric	164	163	89	34	0.20>P>0.10	0.20>P> .10
450	(36.4%)	(36.2%)	(19.8%)	(7.6%)		
Both	25	27	23	7	0.70>P>0.50	0.80>P>0.70
82	(30.5%)	(32.9%)	(28.1%)	(8.5%)		
Gastric carcinoma	439	660	318	148	P<0.001	P<0.001
1565	(28.1%)	(42.2%)	(20.3%)	(9.5%)		
Controls	614	722	426	175		
1937	(31.7%)	(37.3%)	(22.0%)	(9.0%)		

For many years since the discovery of the blood groups by Landsteiner at the beginning of this century, it was generally believed that the blood group substances were of no particular significance in physiological and pathological processes. We have today many evidences that the blood group genes can no longer be regarded as neutral from the viewpoint of natural selection. The essential point of our present knowledge was predicted by Fisher (3) and was first proved to be true by Levine (11). Among persons of different blood groups there must be, to a greater or lesser extent, differences in resistance or susceptibility to diseases or in viability. Considering a population as a whole, the ABO polymorphism is undoubtedly due not only to random genetic drift but also to natural selection. In order to understand the dynamical aspect of this polymorphism in all its complexity, further studies must be carried out vigorously and on a much wider scale; the research will no doubt be of great significance both to human biology and medical science.

ACKNOWLEDGMENTS

The author is indebted to Drs. J. F. Crow at the University of Wisconsin and M. Kimura at the National Institute of Genetics in Mishima for reading the manuscript and for their helpful comments. Thanks are also due to Drs. M. Uetake, T. Tanaka, and K. Fujii at the Tokyo Medical and Dental College for permission to use their unpublished data.

REFERENCES

1. ALLISON, A. C. Aspects of Polymorphism in Man. Cold Spring Harbor Symp. Quant. Biol., **20**:239–55, 1955.
2. COHEN, B. H., and GLASS, B. The ABO Blood Groups and the Sex Ratio. Human Biol., **28**:20–42, 1956.
3. FISHER, R. A. The Genetical Theory of Natural Selection. Oxford: Clarendon Press, 1930.
4. GLASS, B. The Action of Selection on the Principal Rh Alleles. Am. J. Human Genetics, **2**:269–78, 1950.

5. GRUBB, R., and SJÖSTEDT, S. Blood Groups in Abortion and Sterility. Ann. Human Genetics, **19**:183–94, 1955.
6. INOUYE, E. Frequency of Multiple Birth in Three Cities of Japan. Am. J. Human Genetics, **9**:317–20, 1957.
7. ITOH, S. Selection by Incompatibility of Mother and Fetus with Respect to ABO Blood Groups, MN and Q Blood Types. Jap. J. Legal Med., **11**:128–49, 1957.
8. KIHARA, Y. The Problems of Blood Group Incompatibility in Obstetrics and Gynecology. J. Jap. Obst. Gyn. Soc., **6**:449–544, 1954.
9. LEVINE, P. Serological Factors as Possible Causes in Spontaneous Abortions. J. Hered., **34**:71–80, 1943.
10. ———. The Genetics of the Newer Human Blood Factors. Adv. Genetics, **6**:184–234, 1954.
11. LEVINE, P.; KATZIN, E. M.; and BURNHAM, L. Isoimmunization in Pregnancy, Its Possible Bearing on the Etiology of Erythroblastosis Foetalis. J.A.M.A., **116**:825–27, 1941.
12. MATSUNAGA, E. Intra-uterine Selection by the ABO Incompatibility of Mother and Fetus. Proc. Japan Acad., **29**:399–403, 1953.
13. ———. Reproductive Fitness of AB Children from AB × AB Mating. Sapporo M. J., **6**:165–68, 1954.
14. ———. Selektion durch Unverträglichkeit im ABO Blutgruppensystem zwischen Mutter und Fetus. Blut., **2**:188–98, 1956.
15. ———. Selection in ABO Blood Groups. Symposia on Twin Research and Population Genetics. Tokyo: Suppl., Jap. J. Human Genet., **2**:54–58, 1957.
16. MATSUNAGA, E., and ITOH, S. Intra-uterine Selection by the ABO Incompatibility of Mother and Fetus. (II). Proc. Japan Acad., **29**:529–31, 1953.
17. ———. Intra-uterine Selection by the ABO Incompatibility of Mother and Fetus. (III). *Ibid.*, **30**:677–82, 1954.
18. RACE, R. R., and SANGER, R. Blood Groups in Man. 2d ed. Oxford, 1954.
19. REED, E. T. Tests of Models Representing Selection in Mother-Child Data on ABO Blood Groups. Am. J. Human Genetics, **8**:257–68, 1956.
20. ROBERTS, J. A. F. Associations between Blood Groups and Disease. Acta genet., **6**:549–60, 1956.
21. STRANDSKOV, H. H. The Distribution of Human Genes. Sci. Mon., **52**:203–15, 1941.
22. UETAKE, M.; TANAKA, T., and FUJII, K. Relationship between Blood Groups and Diseases among Japanese with Special Reference to Gastric Cancer and Peptic Ulcer (to be published).
23. VAN LOGHEM, J. J., and SPAANDER, J. L'influence de l'incompatibilité du système ABO sur l'antagonisme Rh. Rev. d'Hém., **3**: 276–86, 1948.
24. WOOLF, B. ABO Incompatibility in Haemolytic Disease of the Newborn. Acta genet., **6**:519–20, 1956.

The Relation of the ABO and Rh Blood Groups to Differential Reproduction

BERNICE H. COHEN* and BENTLEY GLASS
The Johns Hopkins University, Baltimore, Maryland

In an earlier study (1), the authors have established the fact that in a Baltimore Rh-incompatibly mated series in which the mothers were nonsensitized (Rh N S series) there is a significantly low sex ratio (.48) in the A offspring of A mothers, whereas there is a significantly high sex ratio (.60) in the B offspring of B mothers. These results agree with those in the series of Rh unselected matings studied by Sanjhvi in Bombay and New York City, and by Johnstone in London. Because we felt the exclusion of the Rh-incompatible matings in which the mother was sensitized might present a bias, after it was learned that ABO-incompatibility offers protection against the occurrence of Rh sensitization, an Rh S series has been studied. In 2998 mother-offspring combinations, the same high sex ratio (.56) in the B offspring of B mothers was again found to occur; but no difference from a normal sex ratio was found in the A/A mother-offspring combinations (.53). The difference between the A/A sex ratios of the two series approaches statistical significance. Whether that difference is spurious or has meaning remains to be discovered. The explanation may, however, lie in the ABO interaction with Rh sensitization which has now been more extensively investigated in the Baltimore data. As Levine (2, 3) and others have shown, maternal-fetal incompatibility with respect to the ABO blood groups, such as occurs when the mother is of blood group O and the fetus of blood group A or B, exerts a protective effect against sensitization to Rh.

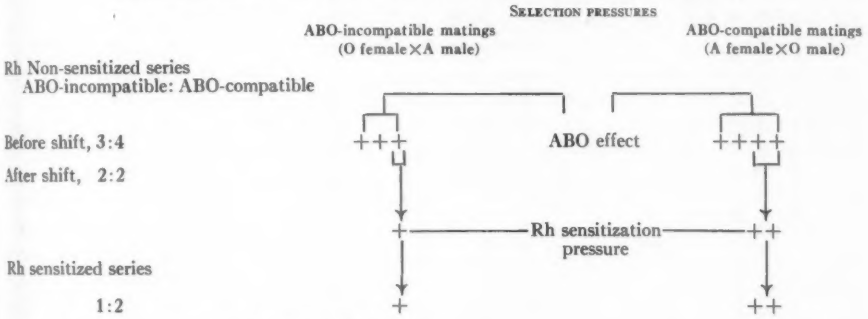
Extensive data from the files of the Baltimore Rh Typing Laboratory have been classified into two series, one in which the mothers are incompatibly mated with respect to Rh but are not sensitized (Rh N S series) and a second series in which the mothers, also incompatibly mated with respect to Rh, are sensitized (Rh S series). In these two series differential reproduction with respect to the ABO blood groups has been studied. Expectancies have been calculated from the general U.S. population, properly weighted for racial composition, as well as from the ABO frequencies found to exist in the parents belonging to the two series actually investigated. The Rh N S and Rh S series are very significantly different from each other, deviating in opposite directions from the expectation for the population. The Rh S series showed a significant deficiency of ABO-incompatible types and an excess of ABO-compatible types in mothers, fathers, offspring, and parent-offspring combinations. Conversely, the Rh N S series had an excess of ABO-incompatible types and a deficiency of ABO-compatible types. For example, in the Rh S series, among 2998 mother-offspring combinations, only 11.84 per cent were ABO-incompatible, as compared with an expected 20.94 per cent ($P < .001$); and among 1383 fertile matings only 25.96 per cent were ABO-incompatible, as compared with an expected 36.08 per cent ($P < .001$). In the Rh N S series, on the other hand,

* Public Health Service Research Fellow of the National Heart Institute.

among 3577 mother-offspring combinations, 22.92 per cent were ABO-incompatible, as compared with an expected 20.90 per cent ($.01 > P > .001$), and among 2313 fertile matings 38.82 per cent were ABO-incompatible, as compared with an expected 36.00 per cent ($.01 > P > .001$). (It should be noted that, if there were no interaction of ABO and Rh effects, the adverse effect of maternal-fetal ABO incompatibility would be expected to lower the frequency of ABO-incompatible offspring and matings in the Rh N S series, and not to increase them or leave them unaffected.) These deviations of the two series in opposite directions result from a preferential shift of ABO-compatible matings from the Rh N S group to the Rh S group, so as to leave a preponderance of ABO-incompatible matings in the Rh N S group (Table 1). This protection against Rh sensitization and hemolytic disease which is conferred by the presence of ABO-incompatibility not only affects the frequencies of Rh alleles, by tending to lessen the adverse selection against the r allele in populations in which this allele is less common than the various $R(D)$ alleles; but, further, the advantage conferred by ABO-incompatibility may react also upon the ABO frequencies, so as to favor A and B in contrast to O. As a consequence of the ABO-Rh interaction, there is a resultant counteraction of opposing selection pressures: the selective disadvantage of ABO-incompatibles in the face of the primary selection pressure against A and B is counteracted by the selective advantage of ABO-incompatibles in the face of the negative Rh selection pressure (Table 2). Thus, within the Rh-incompatibly mated portion of the White population (13 per cent), ABO-incompatibility, instead of acting adversely to A and B, tends to increase their frequencies. Stated another way, double incompatibility at both ABO and Rh loci is superior reproductively to single incompatibility, i.e., Or ♀ × AR ♂ is superior to Ar ♀ × OR ♂. However, since the protection is not complete, the double incompatibility is not superior to, or even equal to, the lack of incompatibility seen in the reciprocal mating AR ♀ × Or ♂. Since the zygotes involved in the double incompatibility are the double heterozygotes, this phenomenon may be described as the superior-

TABLE 1

THE PREFERENTIAL SHIFT IN RH-INCOMPATIBLE MATINGS OF ABO-COMPATIBLE MATINGS IN COMPARISON WITH ABO-INCOMPATIBLE MATINGS



Each "+" represents an equal number of matings.

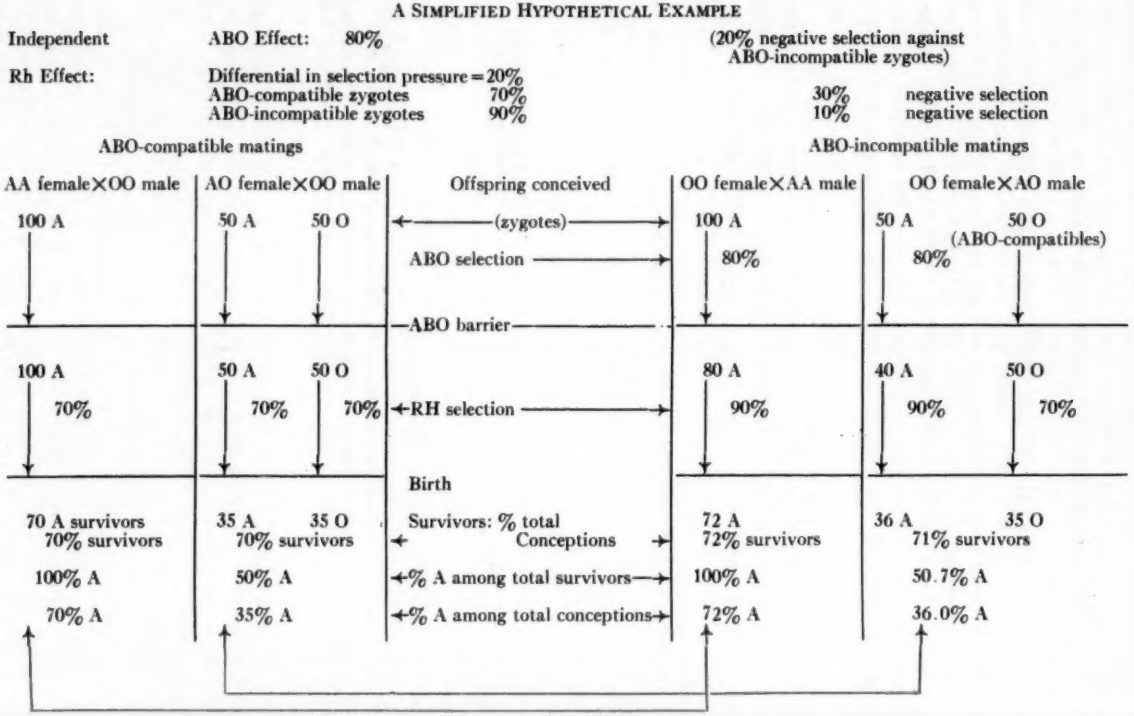
The primary ABO effect is represented by the reduction in number of +'s (fertile matings) representing the ABO-incompatible matings as compared with the ABO-compatible matings (i.e., 3:4).

The differential in Rh sensitization pressure is represented by the number of +'s shifted relative to the number belonging to the mating category before the shift (i.e., 1/3 for O × A matings; 2/4 for A × O matings).

TABLE 2

OPPOSING SELECTION PRESSURES IN RH-INCOMPATIBLE MATINGS

A SIMPLIFIED HYPOTHETICAL EXAMPLE



ity of the double heterozygotes over the single heterozygote. This superiority is, of course, restricted to the presence of the zygote in the incompatibly mated mother.

The protective action of ABO-incompatibility is estimated, from the Baltimore data, to fall between 5 and 15 per cent of the total Rh-incompatible matings, or most probably about 10 per cent. The protection would, therefore, extend to little more than 1 per cent (10 per cent of 13 per cent) of the total matings in the population. It is consequently somewhat unlikely that the protective effect could largely offset the negative selection against A and B owing to the ABO effect in the major, Rh-compatibly mated portion of the population. Nevertheless, the ABO-Rh interaction remains a factor in the complex of selective forces acting on the frequencies of the ABO alleles.

There is some evidence in the Baltimore data that the protection against Rh hemolytic disease which is conferred by ABO incompatibility is not limited to inhibiting or retarding Rh sensitization, but extends also to cases in which the mother has already become sensitized. This may be seen from the following: (*a*) there is a regular rise in the percentage of A offspring in successive pregnancies (O × A matings) within the Rh S series; (*b*) there is a higher pregnancy wastage in O × A (incompatible) matings of the Rh N S series than in A × O (compatible) matings, but a lower pregnancy wastage in O × A matings than in A × O matings of the Rh S series; (*c*) in both series, O × A matings have a significantly higher frequency of spontaneous abortions, attributable to the ABO effect, than do A × O matings; but there is a significantly *lower* frequency of stillbirths, attributable to the Rh effect, in the O × A matings than in the A × O matings of the Rh S series; and, finally (*d*) in both series O × A matings show more live births than A × O matings. If ABO incompatibility indeed confers further protection against Rh hemolytic disease after the sensitization of the mother, then the counteraction of the primary negative selection pressure on the A and B allele frequencies will be greater than was estimated above.

Regardless of the magnitudes of opposing selection pressures in the actual case, this interaction of ABO and Rh incompatibilities is significant in providing a model to illustrate the complexities of selective forces. In human blood group systems, the interaction of two negative selection pressures can, in fact, under certain conditions, yield a positive selection pressure, quite as Timoféeff-Ressovsky (4) long ago demonstrated might occur when certain deleterious mutants in Drosophila are combined.

REFERENCES

1. COHEN, B. H., and GLASS, B. The ABO Blood Groups and the Sex Ratio. Human Biol., **28**:20–42, 1956.
2. LEVINE, P. Serological Factors as Possible Causes in Spontaneous Abortions. J. Hered., **34**:71–80, 1943.
3. ———. The Influence of the ABO System on Rh Hemolytic Disease. Human Biol., **30**:14–28, 1958.
4. TIMOFÉEFF-RESSOVSKY, N. W. Über die Vitalitat einiger Genmutationen und ihrer Kombinationen bei *Drosophila funebris* und ihre Abhängigkeit vom "genotypischen" und vom äusseren Milieu. Ztschr. indukt. Abstamm.-u. Vererblehre, **66**:319–44, 1934.

The Protective Action of ABO Incompatibility on Rh Isoimmunization and Rh Hemolytic Disease—Theoretical and Clinical Implications*

PHILIP LEVINE, M.D.
Ortho Research Foundation, Raritan, N.J.

The combined series of eleven independent studies including 5,146 matings reveal that, in contrast to 35 per cent ABO incompatible matings in random matings, there is an average of 17.7 such matings in Rh hemolytic disease. In all ABO incompatible matings the husband is heterozygous for A or B. Husbands of genotypes AA or BB are excluded. In contrast to 59 matings, 0 Rh-positive husband by AB Rh-negative wife, there was only one reciprocal AB × O mating.

The essential feature for production of Rh antibodies is the survival in the maternal circulation of Rh-positive group compatible fetal red cells for long enough periods to reach the maternal sites of antibody production. If the fetal red cells are ABO incompatible with maternal anti-A or anti-B, they are immediately agglutinated and hemolyzed in the uterine sinuses so that the Rh antigen is not available to provide the antigenic stimulus. These findings provide indirect evidence that in a normal pregnancy the intact fetal red blood cell finds its way into the maternal circulation. More direct evidence is provided by Richardson-Jones who actually demonstrated fetal Rh-positive red cells in the blood of the Rh-negative mother.

The same principles apply also to hemolytic disease due to anti-c, provided that immunization by transfusion is excluded. Here again there are 18 per cent ABO incompatible matings instead of the random 35 per cent. Still additional evidence is provided by the results of immunization of Rh-negative volunteer donors with ABO compatible and incompatible Rh-positive blood (Stern *et al.*).

The chances for production of Rh antibodies are 2.4 times greater in ABO compatible matings than in ABO incompatible matings. (Calculations made with Prof. Howard Levene.)

If fetal red cells enter the maternal circulation, one may assume also a two-way transfer of leukocytes. This may perhaps explain findings of Peer of prolonged survival in 25 per cent of the cases of skin transplants from infant to mother and mother to infant, in contrast to uniform rejection of skin transplants from father to infant and infant to father.

* These findings have been published in the February, 1958, issue of Human Biology and in Memoir No. 86 of the American Anthropologist.

Discussion: Selective Factors in the ABO Polymorphism

J. A. BUCKWALTER, M.D.
Department of Surgery, College of Medicine, State University of Iowa, Iowa City, Iowa

In Chart 1, the associations of statistical significance which have emerged from investigation of selective factors manifest in human disease, in the population of the State of Iowa (Buckwalter *et al.* [2]) related to ABO polymorphism are shown. Data from prospective studies in progress for about a year in no instance have run counter to the findings of these retrospective ones. As a result, differences between patient and control ABO blood type frequencies become more, rather than less, significant.

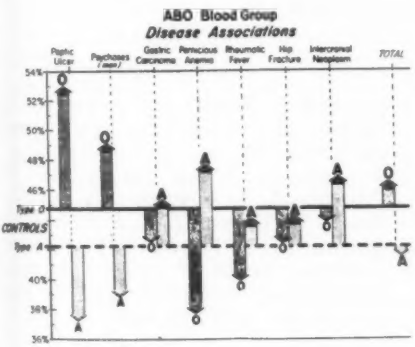


CHART 1.—ABO blood group disease associations

The data from studies of disorders in which equivocal or no statistically significant differences were found, patients from controls (voluntary blood donors), are shown in Chart 2. The findings for carcinoma of the lung and leukemia, although the differences are not statistically significant, are such as to suggest the collection of more data.

One explanation for the continued heterozygosity of the ABO blood groups is a dynamically balanced set of selective factors (Brues [1]), such as the disorders being discussed. Some support for this hypothesis is provided by comparing the composite (6936 patients) blood type frequency curve for the seven patient groups in which statistically significant disease control differences have been found with the composite (3962 patients) curve for the eight patient groups in

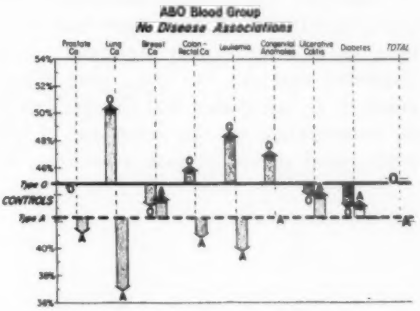


CHART 2.—No ABO blood group disease associations.

which there is no evidence for an association (Chart 3). The differences balance one another, causing the association curve to approach (*a*) the control curve and (*b*) the curve for those diseases in which no association was found.

Population stratification remains a pos-

sible explanation for the ABO blood group disease association. Note that the findings for gastric carcinoma and peptic ulcer in a four South African racial groups may provide a more conclusive answer to the stratification question.

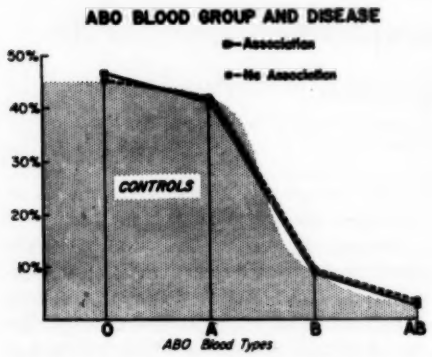


CHART 3.—Composite blood type frequency curves: patients, controls (voluntary blood donors).

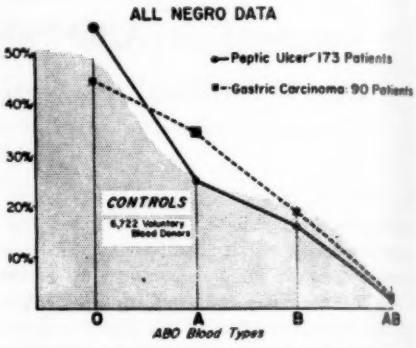


CHART 4.—Blood type frequencies in Negroes: gastric carcinoma and peptic ulcer patients, controls (voluntary blood donors).

Negro population (Buckwalter *et al.* [3]) are similar to those reported for Caucasian populations, i.e., increased incidence of gastric carcinoma in A and peptic ulcer in O type individuals (Chart 4). The Negro data, however, are not statistically significant. Also remember that this was not a pure ethnic group. It is anticipated that the findings of an investigation by the discussant of the ABO blood groups disease association in

REFERENCES

1. BRUES, A. M. Selected and Polymorphism in the ABO Blood Groups. Am. J. Phy. Anthrop., **12**:559–97, 1954.
2. BUCKWALTER, J. A.; TIDRICK, R. T.; KNOWLER, L. A.; WOHLWEND, E. B.; COLTER, D. C.; TURNER, J. H.; RATERMAN, L.; GAMBER, H. H.; ROLLER, G. J.; POLLOCK, C. B.; and NAIFEH, V. K. The Iowa Blood Type Disease Research Project. J. Iowa State M. Soc., **48**:70–81, 1958.

Comment

ERNST MAYR
Museum of Comparative Zoology, Harvard University, Cambridge, Mass.

In an endeavor to study disease-blood group correlations at the pre-reproductive age level, L. K. Diamond and I have recently analyzed over 5,000 admissions to the Children's Hospital at Boston.[1] When subdivided according to diagnosis, the largest subpopulation as a whole and can be used as control sample (Table 1). The ABO composition of admissions for various types of anemias tended to deviate from that of the control group (Table 1). Among "metabolic" anemias and related conditions

TABLE 1

BLOOD GROUP FREQUENCIES AND PERCENTAGES

	O		A		B		AB		
	N	Per cent	N	Per cent	N	Per cent	N	Per cent	TOTAL N
Children's Hospital, Boston:									
Congenital anemias	36	57	20	32	6	9	1	2	63
Metabolic anemias	65	33	92	47	30	15	10	5	197
Congenital heart diseases	583	45	532	41	140	11	48	4	1303
Boston population	55,089	45.8	47,752	39.7	12,990	10.8	4,450	3.7	120,281

χ^2 O against A + B + AB of metabolic anemias vs. congenital heart diseases = 9.63 (P = 0.02).

TABLE 2

BLOOD DISEASES ASCRIBED TO DISORDERS OF METABOLISM, GROWTH, OR NUTRITION

(Children's Hospital, Boston)

Name*	O	A	B	AB	Total
Hypochromic microcytic (iron deficiency) anemia	37	51	21	7	116
Pancytopenic anemia	6	10	3		19
Thrombocytopenic purpura	16	26	4	3	49
Others	6	5	2		13
Total:	65	92	30	10	197

* According to Plunkett, *Standard Nomenclature of Diseases.*

sample consisted of babies with various types of congenital heart defects (N = 1303). These heart cases, separated according to diagnosis or pooled, agreed closely in blood group composition with the Boston (N = 197) there is an excess of A and B (Table 2). Among "congenital" anemias (N = 63) there is a possibility of an excess of O; this includes aplastic, hypoplastic, and hereditary hemolytic anemias. We are continuing this work in an endeavor to increase the size of our samples and to determine the possible statistical significance more clearly.

[1] This investigation was supported by research grant H-2409 from the Heart Institute of the National Institutes of Health.

Discussion on Somatic Cell Genetics

GEORGE KLEIN
Department of Tumor Biology, Karolinska Institutet, Stockholm 60, Sweden

To study the occurrence and the nature of somatic variation under *in vivo* conditions, it is essential to have suitable and specific marker genes. It is also necessary to work out selective systems that enable the detection of specific gene products in single cells and their clonal derivatives.

The histocompatibility genes of the mouse seem to represent the only available system at present that can meet these requirements. By the use of Snell's isogenic resistant (IR) mouse lines, it is possible to restrict genetic variation to one small chromosome segment. For the study of somatic cells, F_1 hybrid mice seem to be particularly suitable, produced by a cross between two IR lines and differing only with regard to the allelic substitution at the H-2 (histocompatibility-2) locus. Allelic differences at this locus are known to constitute an exceptionally strong barrier against homotransplantation. Normal and neoplastic tissues of such F_1 hybrids are usually only transplantable to genetically identical hybrid hosts but not to any of the parental strains, where they provoke a homograft reaction directed against the isoantigens determined by the H-2-allele derived from the opposite parental strain. Mutation of one of the H-2 loci in a heterozygous tumor cell may convey a specific compatibility on its bearer, however, when tested in one of the parental strains, and would thereby become detectable. These considerations have led to the following experiments:

Sarcomas were induced by methylcholanthrene in four IR lines with an A/Sn genetic background and their various F_1 hybrid combinations. Model experiments involving the artificial mixture of known numbers of tumor cells derived from the parental strains and the F_1 hybrids have demonstrated that even a very small randomly admixed compatible cell fraction (with a frequency of about 4×10^{-7}) has an absolute selective advantage in its corresponding host in spite of the fact that the incompatible majority of the population is being destroyed completely by the homograft reaction, and even in cases where compatibility is a matter of a single gene difference. In fact, the homograft reaction directed against the incompatible majority stimulates the growth of the compatible minority.

A series of sarcomas was induced by the same dose of methylcholanthrene in the same tissue of various F_1 hybrid combinations of the four coisogenic resistant lines. The tumors were tested by transplantation to the parental strains and other known genotypes. Seven tumors grew exclusively in their original F_1 hybrid host genotype and did not give rise to variant sublines in any of the parental strains under the conditions of testing. Eight tumors gave rise to a certain proportion of variants capable of growth in *one* of the parental strains. In most cases, the variants were found to be specific for the parental strain of selection upon subsequent testing. No variants were obtained in the other parental strain with these tumors. For each F_1 hybrid genotype, one of the parental strains was clearly preferred to the other as the favored site of variant formation. Six tumors tested gave specific variants in both parental strains. A certain predilection for one of the parental strains, characteristic for the F_1-combina-

tion used, was clearly expressed even in this case, however. Two tumors were "non-specific" and grew in both parental strains and also in foreign genotypes without discrimination. It was not possible to select variant lines with selective compatibility from these strains.

Some tumors and their selected variants were tested by serological methods, involving both hemagglutination and cytotoxic tests, for the presence of certain isoantigens determined by the H-2 system. The findings were closely parallel to the transplantation tests and revealed a specific loss of detectable isoantigens concomitantly with the development of the ability to grow in one of the parental strains.

One tumor (MSWB) that regularly gave rise to a certain proportion of variants in one of the parental strains was examined in detail. Cytological evidence obtained by Bayreuther indicates that the occurrence of variants at different times represents mostly independent events. This was also confirmed by experiments involving the inoculation of very small cell numbers. As a rule, the variants differ both from the original line and from one another with regard to their chromosome cytology. Several lines have individually characteristic marker chromosomes.

In addition to the study of spontaneous variability in populations of tumor cells, histocompatibility genes seem to be excellent markers for investigations on possible recombination mechanisms. Studies of this type are under way.